Heroin and cocaine addiction: a social-cognitive and electrophysiological approach

Thesis (cumulative thesis)

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Abstract

Addiction is a chronic relapsing brain disorder, which is classified as major social, legal and public health problem. Substance use disorders have been postulated to be associated with alterations of neural networks related to reward processing, motivation, memory, and behavioral control, leading to compulsive drug use. Consequently, the value of the abused drug is proposed to be increased, while the value of other (natural) reinforcers is decreased in addiction. However, it is not known, whether neuroadaptations are persistent or reversible after longer periods of abstinence. Furthermore, it is unclear if already recreational drug use is associated with lasting alterations in neurotransmission and whether these alterations are related to deficits in social cognition and interaction. As these issues are important to develop more effective prevention as well as treatment and after-care strategies, we assessed drug cue reactivity in current and long-term abstinent heroin users (Chapter 2), and sensorimotor gating and social cognition in recreational and dependent cocaine user (Chapters 3 and 4). Both heroin and cocaine have been classified as the two drugs bearing the highest harm, with a substantial tendency to induce dependence, and physical as well as social harm. Due to this strong impact on health and society, the following experimental studies were conducted with heroin and cocaine users.

The studies presented in Chapter 2 aimed at investigating implicit and explicit valence of drug cues in heroin users at different stages of abstinence using affective startle modulation (implicit) and valence ratings (explicit). Although drug induced neuroadaptations in brain circuits related to memory have been proposed to be long-lasting, it is unknown whether appetitive effects of heroin-related cues persist after prolonged abstinence. Fifteen current heroin users were measured before and after detoxification therapy. Correspondingly, 15 healthy control participants were tested twice at the same interval of 14 days. Fourteen long-term abstinent heroin users (abstinence duration > 1 year) were assessed additionally. After detoxification and even after prolonged abstinence, heroin cues still exert implicit appetitive effects in heroin users. This confirms that drug-induced adaptations of reward and memory circuits are long-lasting, resulting in a highly stable addiction memory, and therefore an increased susceptibility to relapse.

In the second study (Chapter 3), we investigated if already recreational drug use is associated with alterations in neurotransmission, measured with prepulse inhibition (PPI) of the acoustic

startle response. PPI is a measure of early information processing, more precisely sensorimotor gating, and highly sensitive for manipulations of the catecholamine system. Therefore, we assessed PPI in 64 recreational cocaine users, 29 dependent cocaine users and 66 stimulant-naïve control subjects. Moreover, the influences of craving and attention-deficit/hyperactivity disorder (ADHD) symptoms on PPI were examined, as ADHD is highly comorbid with cocaine use. Recreational and dependent cocaine users showed increased PPI in comparison with control subjects. This increase in PPI was correlated with duration and amount of cocaine use. Moreover, users with a diagnosis of ADHD and strong craving symptoms displayed the highest increases in PPI levels. This suggests that alterations of early information processing, presumably reflecting changes in catecholamine signaling do not only occur after the development of cocaine addiction but already in a non-addicted state. Furthermore, ADHD might be a critical risk factor for cocaine-induced neurochemical plasticity. In addition, PPI might provide a noninvasive and accessible measure to objectively capture craving symptoms.

The study presented in Chapter 4 aimed to investigate potential alterations in social cognition and real-life social functioning in cocaine users. Social abilities are particularly important as social cognition and real-life social behavior have a strong impact on treatment success in psychiatric disorders. Furthermore, pharmacological treatments for cocaine addiction are currently lacking and cognitive-behavioral therapeutic approaches rely, at least in part, on the emotional responsiveness of the patients. To determine social cognitive abilities in cocaine users, we investigated 69 recreational cocaine users, 31 dependent cocaine users, and 68 stimulant-naïve controls by means of video-based and photorealistic tests and related these measures to real-life indicators of social functioning. Our results suggest that recreational and dependent cocaine use is associated with deficits in emotional empathy, whereas dependent cocaine users additionally show impairments in mentalizing. These social cognition deficits are related to real-life social behavior such as a smaller social network and more criminal offences. Furthermore, a younger age of cocaine use onset was linked to pronounced deficits in empathy. These deficits have to be considered to improve the outcomes of therapies.

Taken together, these results indicate that neuroadaptations induced by substance use are long-lasting and therefore trigger relapse even after prolonged abstinence. Moreover, alterations in neurotransmission may already occur in a non-addicted state of drug use. ADHD symptoms might influence the vulnerability for drug-induced plasticity, at least in stimulant users. Furthermore, drug use is associated with deficits in social cognition and social behavior, in particular if drug use already occurred at an early age. The general discussion of this thesis

provides a review of the main results. Considerations regarding the implications for models of addiction, clinical practice and future research are discussed.

Zusammenfassung

Substanzabhängigkeit ist eine chronische neurologische Krankheit, die häufig zu Rückfällen führt. Sie wurde als ernstes gesellschaftliches und juristisches Problem eingestuft, das eine grosse Belastung für das Gesundheitswesen darstellt. Substanzabhängigkeit wird mit Veränderungen neuronaler Netzwerke in Verbindung gebracht, die an Belohnungsverarbeitung, Motivation, Gedächtnis und Verhaltenskontrolle beteiligt sind. Diese neuronalen Adaptationsprozesse unterstützen vermutlich den zwanghaften Substanzkonsum. In Folge dessen kann der Wert der Substanz überhöht verarbeitet werden, während andere (natürliche) Verstärker unterbewertet werden können. Allerdings ist nicht bekannt, ob diese neuroadaptiven Veränderungen bestehen bleiben, oder nach längerer Abstinenz reversibel sind. Ausserdem ist unklar, ob schon gelegentlicher Substanzkonsum mit langfristigen Veränderungen der Neurotransmission assoziiert ist, und ob diese Veränderungen mit Defiziten in der sozialen Kognition einher gehen. Die Beantwortung dieser Fragen ist essentiell, um effektivere Präventions-, Behandlungs-, und Nachsorge-Methoden zu entwickeln. Deshalb untersuchten wir die Verarbeitung von Drogenhinweisreizen bei aktuellen und Langzeit-abstinenten Heroinkonsumenten (Kapitel 2), und sensomotorische Filterfunktionen („sensorimotorisches Gating“) und soziale Kognition bei Gelegenheits- und abhängigen Kokainkonsumenten (Kapitel 3 und 4). Heroin und Kokain wurden als die zwei illegalen Drogen mit dem grössten Schädigungsrisiko eingestuft, da sie ein hohes Abhängigkeitspotential aufweisen, und ein grosses Ausmass an physischem sowie sozialem Leid auslösen können. Wegen dieses grossen Einflusses auf individuelle Gesundheit und Gesellschaft, wurden die folgenden experimentellen Studien mit Heroin- und Kokainkonsumenten durchgeführt.

Die Studien, die in Kapitel 2 vorgestellt werden, untersuchen die implizite und explizite Valenz von Drogenreizen mittels affektiver Schreckreflexmodulation (implizit) und Valenz-Bewertungen (explizit) bei Heroinkonsumenten in verschiedenen Phasen der Abstinenz. Auch wenn angenommen wird, dass neuroadaptive Veränderungen in Folge des Drogenkonsums in den Gedächtnissystemen des Gehirns dauerhaft sind, ist bisher unbekannt, ob Heroinhinweisreize auch nach anhaltender Abstinenz noch appetitive Reaktionen auslösen können. Fünfzehn Heroinkonsumenten wurden vor und nach der Entzugsbehandlung untersucht. Ausserdem nahmen 15 gesunde Kontrollprobanden im gleichen Abstand von 14 Tagen an der Untersuchung teil. Vierzehn langzeitig abstinente Heroinkonsumenten

(Abstinentdauer > 1 Jahr) wurden zusätzlich untersucht. Nach der Entzugsbehandlung und sogar nach anhaltender Abstinenz hatten Heroinhinweisreize immer noch appetitive Effekte für Heroinkonsumenten. Dies zeigt, dass substanzinduzierte adaptive Veränderungen des Belohnungs- und Gedächtnissystems langanhaltend sein können, und zu einem stabilen Drogengedächtnis und damit einer anhaltenden Vulnerabilität für Rückfälle führen.

In der zweiten Studie (Kapitel 3) wurde untersucht, ob schon gelegentlicher Substanzkonsum mit Veränderungen der Neurotransmission verbunden ist. Dies wurde mittels der Präpulsinhibition (PPI) des akustischen Schreckreflexes gemessen. Die PPI ist ein Mass der frühen Informationsverarbeitung, spezifischer des sensomotorischen Gatings, welches äusserst sensitiv für Manipulationen am Katecholamin-System ist. Entsprechend erhoben wir die PPI bei 64 gelegentlichen Kokainkonsumenten, 29 abhängigen Kokainkonsumenten und 66 Kontrollen ohne Stimulanzienkonsum. Ausserdem wurde der Einfluss von „Craving“ (Verlangen nach der Droge) und von Symptomen der Aufmerksamkeits-Defizit/Hyperaktivitätstörung (ADHS) auf die PPI untersucht, da ADHS sehr häuftig komorbid mit Kokainkonsum auftritt. Gelegentliche und abhängige Kokainkonsumenten zeigten im Vergleich zur Kontrollgruppe eine erhöhte PPI. Diese Erhöhung war mit der Dauer und der Menge des Kokainkonsums korreliert. Des Weiteren zeigten Kokainkonsumenten mit einer ADHS Diagnose und Craving-Symptomen die stärkste Erhöhung des PPI-Niveaus. Dies könnte zeigen, dass Veränderungen der frühen Informationsverarbeitung, die vermutlich Änderungen der Katecholamin-Neurotransmission abbilden, nicht nur auftreten, nachdem sich eine Abhängigkeit von Kokain entwickelt hat, sondern schon in einem nicht-abhängigen Zustand vorhanden sind. Ausserdem könnten ADHS-Symptome eine wichtige Rolle als Risikofaktor für Kokain-induzierte neurochemische Plastizität spielen. Zusätzlich könnte die PPI ein non-invasives und gut zugängliches Mass für die objektive Messung von Craving-Symptomen darstellen.

Die Studie, die in Kapitel 4 präsentiert wird, untersucht potentielle Veränderungen der sozialen Kognition und das soziale Funktionieren im Alltagsleben von Kokainkonsumenten. Soziale Fähigkeiten sind besonders wichtig, da sich gezeigt hat, dass soziale Kognition und das soziale Verhalten im Alltag einen starken Einfluss auf den Behandlungserfolg bei psychiatrischen Krankheiten haben. Des Weiteren fehlen bisher effiziente pharmakologische Behandlungsmöglichkeiten der Kokainabhängigkeit und verhaltenstherapeutische Behandlungsmethoden sind, wenigstens zum Teil, auf die emotionale Ansprechbarkeit des Patienten angewiesen. Um sozial-kognitive Fähigkeiten in Kokainkonsumenten zu bestimmen, untersuchten wir 69 gelegentliche Kokainkonsumenten, 31 abhängige Kokainkonsumenten

und 68 Kontrollprobanden mit videobasierten und fotorealistischen Tests und korrelierten diese Masse mit Indikatoren des sozialen Funktionsniveaus im Alltag. Unsere Ergebnisse lassen vermuten, dass gelegentlicher und abhängiger Kokainkonsum mit Defiziten in emotionaler Empathie assoziiert ist. Abhängige Kokainkonsumenten zeigen ausserdem zusätzliche Defizite der mentalen Perspektivenübernahme („Mentalizing“). Diese Defizite der sozialen Kognition waren assoziiert mit dem sozialen Verhalten im Alltagsleben, wie einem kleineren sozialen Netzwerk und mehr begangenen Straftaten. Des Weiteren konnte gezeigt werden, dass der Beginn von Kokainkonsum in jüngeren Jahren mit grösseren Defiziten der Empathie verbunden ist. Diese Einschränkungen sollten in Betracht gezogen werden, um den Erfolg von Therapien zu verbessern.

Zusammengefasst legen diese Ergebnisse nahe, dass neuroadaptive Veränderungen durch Substanzkonsum langanhaltend sind und entsprechend selbst nach längerer Abstinenzzeit einen Rückfall auslösen könnten. Ausserdem können Veränderungen der Neurotransmission schon bei nicht-abhängigen Konsumenten auftreten. ADHS-Symptome könnten des Weiteren die Vulnerabilität für Substanz-induzierte Plastizität bei Stimulanzienkonsumenten erhöhen. Darüber hinaus ist Substanzkonsum mit Defiziten der sozialen Kognition und des sozialen Verhaltens verbunden, vor allem, wenn Drogen schon in jungen Jahren konsumiert wurden. Der Diskussionsteil dieser Arbeit liefert eine Zusammenfassung der Hauptergebnisse. Weiterhin werden Implikationen für Abhängigkeitsmodelle, klinische Arbeit und zukünftige Forschung diskutiert.

1

General Introduction

1.1 Substance use disorders and the brain

Drug dependence is a chronic relapsing brain disorder (Hser *et al.*, 2001) which is defined by compulsive and uncontrolled drug use. Despite serious negative consequences like illness, disrupted social relationships, and the loss of employment, an addicted person's life is centered around the drug of choice and activities related to it (Hyman and Malenka, 2001; Madsen *et al.*, 2012). Many case reports describe people who suffered from life-threatening clinical conditions, depression, utter financial ruin, and social isolation due to drug use but still were not able to quit (Hyman and Malenka, 2001; Volkow *et al.*, 2011a; Waldorf *et al.*, 1991). Furthermore, drug dependence is associated with an enduring, maybe life-long, susceptibility to relapse, often triggered by cues related to the formerly abused drug (Hyman and Malenka, 2001; O'Brien, 1997; Wise and Bozarth, 1987). It is estimated that 22.6 million people in the USA (8.9% of the population) are active (use within the month preceding the survey) illicit drug users (SAMHSA, 2011). In Europe, at least 1.1 million people received treatment for illicit drug use in 2010 (EMCDDA, 2012). Four percent of all deaths of Europeans aged 15-39 are reported to be drug-induced (EMCDDA,2012). Expressed in economic terms, the costs of drug abuse are estimated at 180.9 billion dollars in the USA (Office of National Drug Control Policy, 2004). Therefore, drug misuse and abuse are classified as a major social, legal, and public-health problems (Nutt *et al.*, 2007).

Volkow *et al.* (2011a; 2003; 2011b) proposed a model of the addicted human brain, in order to explain loss of control and compulsive drug intake. According to this model, four brain circuits are engaged in drug abuse and addiction and undergo neuroadaptations with repeated drug use: 1) nucleus accumbens (NAc) and ventral pallidum: reward; 2) orbitofrontal cortex (OFC): motivation/drive; 3) amygdala and hippocampus: memory and learning; 4) prefrontal cortex (PFC) and anterior cingulated gyrus: control (Volkow *et al.*, 2003). These four circuits influence the decisions a person makes by assigning the momentary salience or expected reward to behavioral alternatives. Memories of previous exposures to the situation or the stimulus and internal needs or drives (e.g., hunger or craving) influence the assigned value. Cognitive control engages in the decision to act or not to act. In addiction, the value of the abused drug is proposed to be increased, while the value of other (natural) reinforcers is decreased. This shift might be caused by the higher rewarding properties of drugs: Acute drug administration is associated with 3- to 5- fold higher NAc dopamine (DA) levels than conventional rewards

(Volkow *et al.*, 2003; Wise, 2002). Together with overactive motivation and memory circuits, this weakens the inhibitory control by the PFC and results in a positive-feedback loop leading to compulsive drug taking (Volkow *et al.*, 2003) and the transition from recreational to addicted drug use (Madsen *et al.*, 2012) **(Figure 1)**.

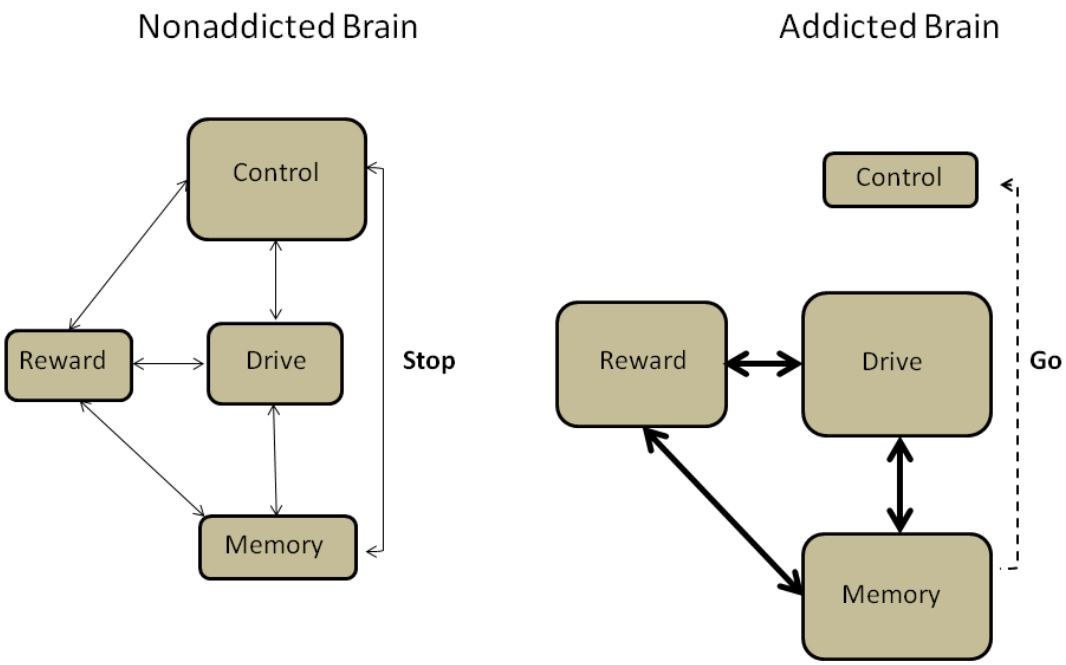

Figure 1. Model by Volkow *et al.* (2011a; 2003; 2011b) suggesting a network of four brain circuits contributing to addiction: reward, motivation/drive, memory/conditioning, and control. In the addicted brain, the value of the drug of choice is enhanced in reward, motivation and memory circuits. This overcomes the inhibitory control of the PFC and triggers a positive-feedback loop leading to compulsive drug use. Several neurotransmitters are involved in theses neurodaptations: dopamine, glutamate, GABA, norepinephrine, corticotrophic releasing factor, and opioid receptors. Adapted from Volkow *et al.* (2011a; 2003; 2011b).

This model is in line with theories of addiction memory, which presume shared mechanisms between neuroplasticity, learning, memory, and drug addiction (Berke and Hyman, 2000; Hyman and Malenka, 2001; Hyman *et al.*, 2006; Kelley, 2004; Robbins and Everitt, 2002; White, 1996). Neural networks engaged in plasticity and consolidation of experiences (dopaminergic and glutamatergic systems) are suggested to be influenced by repeated drug administration and drug-context pairings (Kelley, 2004). In accordance with this, the incentive-sensitization

theory of addiction (Robinson and Berridge, 1993) suggests that avoidance of withdrawal symptoms is not the most important factor in the maintenance of drug use as proposed by negative reinforcement theories (Solomon and Corbit, 1974; Tiffany, 1990). Neuroadaptations resulting from repeated drug use are suggested to render the DA system hypersensitive (sensitized) to drugs and drug-associated stimuli. This sensitization turns the act of drug taking and drug cues into potent incentives which induce a strong feeling of wanting to use the drug ("craving"). Previous studies have provided support for this model by showing that drug associated cues are processed as appetitive by dependent smokers (Dempsey *et al.*, 2007; Geier *et al.*, 2000) and alcohol users (Mucha *et al.*, 2000).

Furthermore, this model has implications for social cognition in addicted persons. Sensitivity to social reinforcers such as social interaction might be reduced, while the value of the drug of choice is increased (Volkow *et al.*, 2003). Moreover, neural networks described to be affected by drug use (e.g., frontal areas and the DA system) also engaged in social cognition and social reward (Abu-Akel and Shamay-Tsoory, 2011; Fan *et al.*, 2012; Gallagher and Frith, 2003; Krach *et al.*, 2010). This may have a negative impact on general social competence and might explain why social threats such as imprisonment or familial problems may fail to detain drug addicted people from using the drug (Volkow *et al.*, 2011a; 2003; 2011b).

However, some open questions remain, which we aim to address in the experimental studies of this thesis (Chapters 2-4): Firstly, although drug induced neuroadaptations in brain circuits related to memory have been proposed to be long-lasting, it is unknown whether appetitive effects of heroin-related cues are stable after prolonged abstinence. Therefore, the studies presented in Chapter 2 investigate the implicit valence of drug-related stimuli in different states of abstinence in current and former heroin addicted patients. Secondly, it is not clear if recreational drug use is already associated with alterations in neurotransmission. In Chapter 3, we therefore investigated if alterations in sensorimotor gating - associated with catecholamine neurotransmission - can already be measured in a non-addicted state of cocaine use. Thirdly, it is not known, if social cognition deficits predicted by the model by Volkow *et al.* (2011a; 2003; 2011b) are existent and can be measured in cocaine users. Thus, in Chapter 4, we studied social cognition in recreational and dependent cocaine users and investigated if potential deficits are related to real-life social functioning.

1.2 Heroin and cocaine use

In the previous paragraph, it was described that the aim of this thesis is to gain more insight into the neural, behavioral and social alterations which might accompany drug use in different states of addiction (non-dependent use, dependence, and abstinence). This knowledge may help to improve prevention, treatment and after-treatment strategies. Heroin and cocaine have been classified as the two drugs with the highest harm score, when physical harm, the tendency to induce dependence, and social harm (the effect of drug use on families, communities, and society) are factored into the rating (Nutt *et al.*, 2007). Due to this strong impact on health and society, the experimental part of this thesis (Chapter 2-4) focuses on users of heroin and cocaine. In the following paragraphs, heroin and cocaine use (1.2.1), their acute effects and mode of action (1.2.2), and neuroadaptive changes associated with dependent use (1.2.2 and 1.2.4) will be further discussed. In Chapter 1.3 methodical considerations related to the experimental studies of this thesis (Chapters 2-4) will be explained. After that, the study samples of Chapter 2 and the Zurich Cocaine Cognition Study (ZuCo²St), which incorporates the studies presented in Chapter 3 and 4, will be introduced.

1.2.1 Prevalence of heroin and cocaine use

The use of heroin has been stable from 2005 to 2010 (EMCDDA, 2012). 1.4 million problem opioid users were reported in the European Union and Norway in 2010 (EMCDDA, 2012). More than half of treatment seeking drug users report opioides as their primary drug in Europe. Furthermore, heroin is responsible for the greatest part of morbidity and mortality related to drug use in the European Union (EMCDDA, 2011).

Cocaine is the second most prevalent illegal drug after cannabis in Europe (EMCDDA, 2011; 2012) and the USA (HHS, 2011). Lifetime prevalence for cocaine use is estimated at 6.3% in Europe among 15 to 34 year olds (EMCDDA, 2012) and 13.3% in the USA among 18 to 25 year olds (SAMHSA, 2011). As not everyone exposed to drugs will undergo the transition to dependence (genetic, developmental and environmental factors also influence this transition (Volkow and Li, 2005)), recreational cocaine use is reported to be a prevalent consumption pattern (EMCDDA, 2011).

1.2.2 Acute effects of heroin and cocaine

The acute effects of heroin (diacetylmorphine) are ascribed to the stimulation of opioid receptors (mu, delta, and kappa) and the indirect increase of DA levels by reducing the inhibitory effect of GABA in the ventral tegmental area (Fernandez-Serrano *et al.*, 2011; Johnson and North, 1992). Repeated use of heroin induces tolerance and physical dependence associated with severe withdrawal symptoms. Before tolerance is developed, acute heroin administration produces feelings of euphoria often referred to as “rush”, sedation, and tranquility (Cami and Farre, 2003).

Acute administration of cocaine increases the synaptic levels of DA, serotonin, and norepinephrine (NE) via a blockade of the corresponding presynaptic transporters (Ritz *et al.*, 1990). The acute effect of cocaine leads to an intense feeling of euphoria (“high”), which is mainly associated with DA transporter (DAT) occupancy (Bolla and Cadet, 2007). Further effects of acute cocaine use are enhanced attention and sensory alertness (to light, sound and touch), accelerated thinking, increased self-confidence, and suppression of the need to eat or sleep (Brownlow and Pappachan, 2002). The phase of euphoria only lasts up to one hour, followed by a period of craving, depression, low energy, and impaired cognition (“crash”) if no more cocaine is administered (Gawin and Kleber, 1986).

1.2.3 Neuroadaptive changes and cue reactivity in dependent heroin users

Opiate-dependent patients have been reported to show alterations in brain areas which are part of the addiction networks proposed by Volkow *et al.* (2011a; 2003; 2011b). Studies using voxel-based morphometry (VBM) found reductions in gray matter in prefrontal, temporal, and cingulate cortices (Lyoo *et al.*, 2006; Upadhyay *et al.*, 2010; Wang *et al.*, 2012). Using diffusion tensor imaging (DTI) altered white matter integrity in reward circuits and memory-related areas has been reported in heroin-dependent patients undergoing methadone maintenance treatment (Lin *et al.*, 2012). Furthermore, altered white matter integrity was correlated with worse scores in memory function (Lin *et al.*, 2012).

Supporting theories of addiction memory (Berke and Hyman, 2000; Hyman and Malenka, 2001; Hyman *et al.*, 2006; Kelley, 2004; Robbins and Everitt, 2002; White, 1996), enhanced attentional processing of heroin cues has been reported for heroin dependent patients using methods like the Stroop task and electrodermal responses (Franken *et al.*, 2000; Lubman *et al.*,

2008). This attentional bias has been associated with alterations in DA functioning (Franken *et al.*, 2004). Enhanced cue reactivity has also been found to be a predictor for future heroin use and relapse after treatment (Fatseas *et al.*, 2011; Lubman *et al.*, 2009; Marissen *et al.*, 2006). Therefore, sustained appetitive value of heroin cues might contribute to the high relapse rates in heroin addiction (Carter and Tiffany, 1999; Fatseas *et al.*, 2011). However, so far, the implicit valence of drug-related cues has not been investigated in heroin users before and after detoxification and withdrawal therapy, and after long-term abstinence from opioids. Therefore, the study presented in Chapter 2 focuses on this issue.

1.2.4 Neuroadaptive changes in dependent cocaine users and associations with behavior

Chronic cocaine use has also been shown to be associated with neuroadaptations in brain areas which are part of the addiction networks proposed by Volkow *et al.* (2011a; 2003; Volkow *et al.*, 2011b). In particular, structural and functional alterations have been reported in the frontal lobe, i.e. the orbitofrontal cortex (OFC) and the prefrontal cortex (PFC), the anterior cingulate cortex (ACC), temporal cortices including insula, and striatal regions of chronic cocaine users (Bolla *et al.*, 2004; Ersche *et al.*, 2011; Franklin *et al.*, 2002; Hanlon and Canterberry, 2012; Kuhar and Pilotte, 1996; Makris *et al.*, 2008; Volkow *et al.*, 1992). It has been shown that a longer duration of cocaine use is associated with greater grey matter reductions in the OFC, ACC, and the insular cortex (Ersche *et al.*, 2011). Previous studies also reported reduced cortical thickness for the dorsolateral prefrontal cortex (DLPFC) and temporal cortices in dependent cocaine users compared with controls (Franklin *et al.*, 2002; Makris *et al.*, 2008). Furthermore, less resting-state functional connectivity has been reported in frontolimbic systems in cocaine dependent subjects (Verdejo-Garcia *et al.*, 2012). Using Positron Emission Tomography (PET), it has repeatedly been shown that chronic cocaine use is associated with reduced striatal DA D2 receptor availability and blunted striatal DA release (Martinez *et al.*, 2004; Martinez *et al.*, 2007; Volkow *et al.*, 1993; Volkow *et al.*, 1997b). These dysfunctions in the DA system of dependent cocaine users have been associated with extensive consequences including craving, impulsive behavior, loss of control over drug intake, and relapse (Martinez *et al.*, 2007; Volkow *et al.*, 1997a; Volkow *et al.*, 1997b). Nevertheless, it has not been investigated yet, if these dysfunctions only occur in a cocaine-addicted state or if already recreational cocaine users show alterations in neurotransmission. Thus, the study presented in Chapter 3 aims to investigate this question.

Furthermore, Volkow and Li (2004) suggested that adaptations reported in dopaminergic circuits turn cocaine users less sensitive to dopamine increases induced by natural reinforcers. Previous studies support this idea by reporting that cocaine users show reduced sensitivity to secondary reinforcers such as monetary reward (Goldstein *et al.*, 2007a; 2007b). Additionally, dysfunction of the OFC of chronic cocaine users has been associated with impaired descision-making abilities (Bolla *et al.*, 2003). Moreover, altered functioning of the PFC and ACC have been related to deficits in impulse control (Bolla *et al.*, 2004). These brain areas and functions have also been shown to play a role in social cognition (Abu-Akel and Shamay-Tsoory, 2011; Volkow *et al.*, 2011a). However, studies on social cognition in cocaine users are scarce. Therefore, the data shown in Chapter 4 evaluate empathy, metalizing and real-life social functioning in cocaine users.

1.3 Methodical considerations

In the previous paragraphs, it was explained that the experimental part of this thesis is designed to examine presumed lasting changes in cue-reactivity after therapy and long-term abstinence, to explore if alterations in neurotransmission already occur in a non-addicted state of drug use, and if drug use is associated with changes in social functioning. In order to investigate these issues, we conducted the studies reported in detail in Chapters 2-4 using the following methods: Affective startle modulation (Chapter 2), prepulse inhibition (PPI), startle reactivity, and habituation (Chapter 3), and video- and photo-based behavioral tests of social cognition, in combination with real-life measures of social functioning (Chapter 4). These methods are briefly described in the following paragraphs.

1.3.1 Affective startle modulation

Affective startle modulation is a well-established non-subjective method to assess the implicit valence of stimuli and in particular drug-associated cues (Grillon and Baas, 2003). The acoustic startle reflex (ASR) is induced by a sudden and intense auditory environmental stimulus and is most reliably measured as a rapid closure of the eyelids. It occurs reflexively as a contraction of the orbicularis oculi muscle 30-50ms after the onset of the startling stimulus (Lang *et al.*, 1990) and can be recorded using electromyography (EMG). A primary startle circuit regulates the ASR by a three-synapse pathway in the brainstem: Over the cochlear nerve startling input reaches the nucleus reticularis pontis caudalis (PnC) and is then sent to spinal motor neurons (Koch, 1999). This eye-blink reflex provides an objective measure of a person's affective reactions and motivational status towards stimuli, as there is a linear relationship between the affective valence of a foreground stimulus and startle magnitude: The reflex is inhibited when an individual is exposed to pleasant pictures and is augmented when the person views unpleasant pictures (Lang *et al.*, 1990, 1998). This affective modulation of the startle reflex is ascribed to a motivational priming effect: The startling noise elicits a negative, defensive reflex, which probably serves a protective function, such as avoiding organ injury (Graham, 1975). Negative foreground stimuli activate the defense motive system and induce an emotional aversive state. This results in an augmentation of the defensive startle reflex. On the other hand, positive

affective priming weakens the aversive state and therefore inhibits the startle reflex and reduces the startle magnitude (Lang *et al.*, 1998).

The affect-modulated startle method has been repeatedly used in addiction research. Photographs of drug paraphernalia or drug intake rituals are used as foreground stimuli and the suppression of the startle response by drug-related scenes relative to neutral scenes (referred to as cue-related startle suppression, CSS) is measured. In this context, objective measures are particularly important, as the reaction to drug-related cues might be in part unconscious (Tiffany and Drobes, 1990). In general, these studies confirmed incentive theories of drug addiction (discussed in Chapter 1.1). CSS and therefore appetitive effects of drug cues have been reported in addicted users of alcohol or nicotine while viewing drug-related cues (Cinciripini *et al.*, 2006; Dempsey *et al.*, 2007; Geier *et al.*, 2000; Mucha *et al.*, 2000). However, it is not known, whether heroin-related cues still elicit implicit appetitive effects after drug withdrawal and therapy and after long-term abstinence. Therefore, in the studies described in Chapter 2, affective startle modulation was used to assess implicit affective reactions in to heroin-related cues in addition to explicit ratings of valence and craving in 15 current heroin users before and after detoxification therapy, 14 long-term abstinent heroin users and 15 controls. We hypothesized that heroin cues sustain their incentive value even after longer periods of therapy and abstinence. This would help to explain the high relapse rates in heroin users (O'Brien, 1997) and support addiction memory theories which propose long-lasting changes in the memory circuits of drug users.

1.3.2 Prepulse inhibition

PPI of the ASR is a form of startle plasticity, which is considered as a translational measure of sensorimotor gating. Sensorimotor gating describes an early information processing mechanism and a universal preattentional filter function by which irrelevant stimuli are gated out, so that a person can focus on the most important aspects of the environment (Braff and Geyer, 1990; Braff *et al.*, 2001). PPI refers to the natural, unlearned attenuation of the startle response when the intense startling stimulus (pulse) is preceded by a weaker non-startling stimulus (prepulse) by 30 - 500 ms (Graham, 1975). PPI has been reported across a large variety of species: besides others in fish (Burgess and Granato, 2007), rodents (Hoffman and Searle, 1965), non-human primates (Linn and Javitt, 2001), and humans (Braff and Geyer,

1990). In humans, ASR and PPI are measured by EMG of the orbicularis oculi facial muscle, as described in 1.3.1.

PPI is regulated by sequential and parallel neural connections of a cortico-striato-pallido-pontine (CSPP) circuit, which involves the PFC, the ventral striatum including the NAc, the ventral pallidum, and the ventral tegmentum (Koch, 1999; Swerdlow *et al.*, 1999). This CSPP circuit converges with the primary startle circuit (see 1.3.1) at the PnC (Swerdlow *et al.*, 1999). DA and NE have been shown to play a crucial role in the regulation of PPI (Alsene *et al.*, 2011; Zhang *et al.*, 2000). Consequently, PPI has been reported to be highly sensitive to changes in catecholamine neurotransmission (Braff *et al.*, 2001; Zhang *et al.*, 2000). Disorders associated with abnormalities in the CSPP circuitry that regulates PPI are accompanied by alterations in PPI. For example, deficient PPI has been reported in patients with Huntington's disease (Swerdlow *et al.*, 1995), Tourette's syndrome (Castellanos *et al.*, 1996), and schizophrenia (Braff *et al.*, 1992); diseases which are presumably associated with dysregulated DA neurotransmission. Antipsychotic medication has been shown to normalize PPI deficits in schizophrenic patients (Csomor *et al.*, 2009; Kumari *et al.*, 2002; Leumann *et al.*, 2002; Quednow *et al.*, 2006). Especially alterations in the ventral part of the mesostriatal DA system (Braff *et al.*, 2001; Zhang *et al.*, 2000) and the thalamocortical and ventral forebrain NE networks are associated with changes in PPI (Alsene and Bakshi, 2011; Alsene *et al.*, 2011; Oranje and Glenthoj, 2012; Oranje *et al.*, 2004). Therefore, PPI classifies as an inexpensive and non-invasive measure (as compared to PET) to investigate neurochemical alterations in the CSPP circuit.

In rats, acute cocaine administration has been shown to reduce PPI (Martinez *et al.*, 1999), but lasting effects of repeated drug use are not well studied. CSPP circuits regulating PPI, and the reward system (shown to be altered in cocaine users) overlap in the ventral striatum. Therefore, PPI is supposed to be an appropriate measure to capture alterations of catecholamine functioning in cocaine users, which have been associated with craving, loss of control over drug intake, and relapse (Martinez *et al.*, 2007; Volkow *et al.*, 1997a; 1997b). Therefore, the study described in Chapter 3 aims to investigate 1) if dependent cocaine users show alterations in PPI that are known to be related to changes in neurotransmission 2) if already recreational drug use is associated with alterations in neurotransmission measured with PPI and 3) if ADHD symptoms which are highly comorbid with cocaine dependence and abuse and craving (Perez de Los Cobos *et al.*, 2011), influence PPI.

1.3.3 Social cognition

The model of addiction proposed by Volkow *et al.* (2011a; 2003; 2011b) (see Chapter 1.1) implies that drug use should have consequences for social cognition, as sensitivity to social reinforcers might be reduced. Furthermore, dependent cocaine users show neurochemical and functional changes in brain areas related to social cognition (Hanlon and Canterberry, 2012; Volkow *et al.*, 2011a). Moreover, social cognition is modulated by the DA system (Abu-Akel and Shamay-Tsoory, 2011), which is altered in cocaine users, as will be demonstrated in Chapter 3.

Social cognition in drug users is particularly important, as social abilities and social support are critically involved in the onset of drug use (Ramirez *et al.*, 2012; Shortt *et al.*, 2007) and treatment success in addiction disorders (Homer *et al.*, 2008; Volkow *et al.*, 2011a). Pharmacological treatment approaches for cocaine addiction are currently lacking (O'Brien, 2005) and treatment approaches such as cognitive behavioral therapy rely, at least partly, on the emotional responsiveness of the patients (Moos, 2007). Therefore, a characterization of social cognition deficits in cocaine users is important to improve the impact of treatment strategies for cocaine addiction. However, studies on social cognition in cocaine users have been lacking so far. Therefore, the study presented in Chapter 4 aims to investigate social cognition and real life social functioning in dependent and recreational cocaine users.

As measures of social cognition, the Multifaceted Empathy Test (MET) (Dziobek *et al.*, 2008), the Movie for the Assessment of Social Cognition (MASC) (Dziobek *et al.*, 2006), and the Reading the Mind in the Eyes Test (RMET) (Baron-Cohen *et al.*, 2001) were applied. The MET and MASC are recently developed tests, which rely on photo-realistic and video-based stimulus material and therefore offer more ecological validity than for example questionnaires or text-based tests.

The MASC is a naturalistic, video-based and PC-assisted test of mentalizing, which has been shown to be more sensitive than other mindreading tests (Dziobek *et al.*, 2006), such as the strange stories task (Happe, 1994), RMET (Baron-Cohen *et al.*, 2001), and basic emotion recognition (Ekman and Friesen, 1971). Participants are asked to watch a short movie about an everyday life situation (friends having dinner) and make inferences about the video characters' mental states. This requires the understanding of different mental states such as emotions, thoughts, and intentions, and social cognition concepts, such as false belief, faux pas, metaphor, and sarcasm in an everyday life situation. Furthermore, valence, extent and quality

of language, gestures, and facial expressions are varied. Therefore, the test covers a broad range of mental states that have to be inferred (Dziobek *et al.*, 2006). The MASC has been reported to detect even subtle mindreading difficulties for example in healthy individuals of normal IQ (Hassenstab *et al.*, 2007; Smeets *et al.*, 2009), and patients with schizophrenia (Montag *et al.*, 2011), borderline personality disorder (Preissler *et al.*, 2010), and autism (Dziobek *et al.*, 2006). The test has a good internal consistency with Cronbach's α=0.84 and has been shown to be very stable over time with an interclass coefficient ICC = 0.97 over an average interval of 4.6 months.

The MET requires participants to view photographs of people in emotionally charged situations and infer the mental state of the person in the photo, rate their empathic concern for the person, and judge their arousal while viewing the picture (Dziobek *et al.*, 2008). The stimuli depict everyday life situations conveying information on emotional mental states via facial expression, body language, and context. The MET is based on the multidimensional model of empathy (Blair, 2005; Davis, 1983), and therefore is feasible to differentiate two facets of empathy: cognitive and emotional empathy. The cognitive aspect refers to the ability to understand another person's mental state and emotion, without necessarily experiencing the same emotion or being in an emotional state (Baron-Cohen and Wheelwright, 2004; Walter, 2012). Thus, the concept of cognitive empathy overlaps with affective Theory of Mind (ToM) (Walter, 2012) and mentalizing (Frith and Frith, 2003). Emotional empathy refers to the emotional response of a person to another person's emotional state and therefore, the ability to feel what someone else feels (Mehrabian and Epstein, 1972). Thus, the MET is designed to create a more specific profile of empathic abilities than unidimensional tests and questionnaires of empathy used in previous studies (Baron-Cohen and Wheelwright, 2004; Blair, 1999). Moreover, through the use of photorealistic stimuli it aims to better capture empathic abilities than multidimensional empathy questionnaires (e.g., the Interpersonal Reactivity Index (Davis, 1980)) which hold lower ecological validity (Dziobek *et al.*, 2008). The MET has been reported to detect deficits in cognitive empathy in patients with autism (Dziobek *et al.*, 2008), and deficits in emotional empathy in patients with narcissistic personality disorder (Ritter *et al.*, 2011). Internal consistency of the MET scales ranges from Cronbach's α=0.71 to α=0.92 (Dziobek *et al.*, 2008).

When completing the RMET participants are asked to infer the mental state of a person from a photograph which depicts the eye region only (Baron-Cohen *et al.*, 2001). The participants are supposed to choose one out of four mental state descriptors that describes the person's

feelings or thoughts best. Amongst others, autistic (Baron-Cohen *et al.*, 2001) and schizophrenia patients (Kother *et al.*, 2012) showed impaired abilities on mental state perception in the RMET.

To assess real-life social functioning and behavior, the Social Network Questionnaire (SNQ) was conducted and the number and type of committed criminal offences was provided by the participants. The SNQ is a questionnaire designed to calculate the size of a persons' social network. Furthermore, the experienced strain, and practical and social support by this network can be evaluated. It is based on the multidimensional social contact circle interview (Linden *et al.*, 2007).

Preliminary studies disclosed that dependent cocaine users might have difficulties in understanding, management, and regulation of emotions (Fox *et al.*, 2007; Fox *et al.*, 2011; Kemmis *et al.*, 2007), and a 22-fold increased risk of antisocial personality disorder (Rounsaville, 2004). However, social cognitive abilities such as mentalizing and empathy have not been investigated in cocaine users so far. Therefore, the study presented in Chapter 4 investigates these abilities using the MASC, MET, and RMET and relates the outcomes to real life social functioning and behavior measured with the SNQ and the reported criminal offences.

1.4 Study samples

1.4.1 Current and long-term abstinent heroin users

Chapter 2 presents two studies on affective startle modulation in current and long-term abstinent heroin users. The fist study was carried out with 15 heroin-dependent subjects, who were enrolled in a residential detoxification program at the Department of Psychiatry of the University of Bonn, Germany. These participants underwent detoxification therapy with methadone, which was decreased within 8-12 days of inpatient treatment and had never received methadone maintenance prior to this therapy.

The 15 long-term abstinent heroin users recruited for study II took part in a longitudinal-investigation on the genetic influence on vulnerability to heroin dependence (Xu *et al.*, 2004). Only heroin users who stated to be abstinent for at least one year were considered for this study. Abstinence was controlled by urine toxicology. None of these participants received methadone or heroin maintenance therapy. The control group was recruited from the general population.

1.4.2 The Zurich Cocaine Cognition Study (ZuCo^{2}St)

The studies presented in Chapters 3 and 4 were part of a larger longitudinal investigation on the effects of cocaine use – the Zurich Cocaine Cognition Study (ZuCo^{2}St). The study is designed to assess the impact of cocaine use on different measures of social and non-social cognition, impulsivity, and decision-making. Furthermore, the genetic basis of cocaine addiction, as well as glutamatergic changes measured with PET and structural and functional alteration measured with (functional) Magnetic Resonance Imaging (MRI) in cocaine users are investigated. Chapter 3 focuses on the early information processing and Chapter 4 on social cognition part of the ZuCo²St. Further data are discussed in different theses or are part of upcoming publications.

One distinctive feature of the ZuCo^{2}St is the inclusion of a non-addicted user group in addition to dependent users. Even though a substantial part of cocaine users use the drug in a recreational manner (EMCDDA, 2011), very little is known about this group, even though non-addicted users offer the possibility to investigate factors that might influence the transition to

dependence. Furthermore, this group holds the following advantages: They are 1) less burdened by psychiatric comorbidities (Smith *et al.*, 2009), 2) less likely medicated with psychotropic drugs, and 3) display a reduced amount of polytoxic drug use.

Another exclusive aspect is the application of comprehensive psychiatric diagnostics and hair-toxicology offering the possibility to screen the drug use of up to six months before the testing. Out of 1063 requests via email or telephone 804 potential participants completed an initial telephone screening, whereof 240 fulfilled the inclusion criteria and participated in the study. Hair toxicology and urine samples were then applied to validate self-reports of drug use. Due to untruthful reports or the fulfilling of exclusion criteria, 46 participants had to be excluded, which resulted in a final sample of 108 cocaine users and 86 control subjects. Therefore, this sample is very well-described with little co-substance use and little psychiatric comorbidities, which is unique so far. Further details are presented in Chapters 3 and 4.

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2

Sustained incentive value of heroin-related cues in short- and long-term abstinent heroin users

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KHP, MW, and BBQ contributed to data analysis and writing of the manuscript. BBQ, KH, and MW contributed to the study design and development of the startle paradigm. AKR contributed to the writing of the manuscript. CS contributed to study design, data acquisition, and writing the manuscript. JN contributed to data acquisition and writing of the manuscript. IF and NP contributed to preprocessing of the data, data analysis, and writing the manuscript. PEF recruited and treated the patients and contributed to the writing of the manuscript.

2.1 Abstract

Models of addiction and addiction memory propose that drug-associated cues elicit incentive effects in drug users, which play an important role in maintenance of drug use and relapse. Incentive effects have been demonstrated for smoking and alcohol-related cues but evidence for heroin-related cues has been inconclusive. Furthermore, it is unknown whether appetitive effects of heroin-related cues persist after prolonged abstinence, although heroin addiction is known to have high relapse rates. Therefore, we investigated implicit and explicit valence of heroin-related cues in dependent users at different stages of abstinence using affective startle modulation. In *Study I*, 15 current heroin users were measured before and after detoxification. Correspondingly, 15 healthy control participants were tested twice at an interval of 14 days. In *Study II,* 14 long-term abstinent heroin users were additionally measured in a single session. Implicit processing of drug-related stimuli was assessed using affective startle modulation by pictures of heroin and smoking scenes. Explicit reactions were measured using ratings of valence and craving. In contrast to controls, heroin-dependent participants showed a clear reduction of startle response during heroin-related pictures ($p<0.05$). Detoxification did not significantly change their startle responses to heroin-cues. No difference between non-detoxified current and long-term abstinent heroin users was found in implicit reactions to heroin-cues, whereas explicit measures differed between both groups (all $p<0.05$). After detoxification and even after prolonged abstinence, heroin cues still exert implicit appetitive effects in heroin users. This implies that drug-induced adaptations of reward circuits are long-lasting, resulting in a highly stable addiction memory.

2.2 Introduction

Drug dependence, opiate dependence in particular, can be considered as a chronic relapsing disorder (Hser *et al.*, 2001). Models of addiction propose that cue reactivity (i.e. elicitation of conditioned responses on psychological, physiological, and behavioral levels by drug-associated stimuli) is of particular importance in the maintenance of drug use and relapse (Carter and Tiffany, 1999; Fatseas *et al.*, 2011). Traditional theories of drug cues hold that drug-associated stimuli evoke conditioned reactions that trigger withdrawal-like and aversive responses (Koob *et al.*, 1997; Wikler, 1973). More recently, there is evidence that drug-associated cues may rather be processed as appetitive leading to the same behavioral and neurobiological responses like the drug itself (Dempsey *et al.*, 2007; Geier *et al.*, 2000; Mucha *et al.*, 2000). Moreover, the incentive-sensitization theory proposes that drug consumption produces incremental neuroadaptations in the mesolimbic dopamine pathways, rendering them hypersensitive to drugs and drug-associated stimuli (Robinson and Berridge, 2000). This sensitization turns the act of drug taking and stimuli associated with it into powerful incentives that produce a strong feeling of "craving" for the preferred drug. Craving commonly refers to the subjective experience of wanting to use a drug (Tiffany and Wray, 2012) and the urge to re-experience the effect of a psychoactive substance (UNDCP/WHO, 1992).

This is in line with multiple theories of addiction memory, which propose shared mechanisms between memory, learning, and addiction (Berke and Hyman, 2000; Hyman and Malenka, 2001; Hyman *et al.*, 2006; Kelley, 2004; Robbins and Everitt, 2002; White, 1996). Repeated pairings of drugs and environment are supposed to produce long-term, maybe permanent, neuroadaptive effects in motivational networks that lead to the establishment of compulsive drug-seeking habits (Kelley, 2004; Robbins and Everitt, 2002). These associations and the craving elicited by drug-related stimuli might be in part unconscious (Tiffany, 1990). Drug memories are therefore supposed to be long-lasting and implicit. This assumption is consistent with high relapse rates among heroin users following treatment (O'Brien, 1997).

A well-established non-subjective method to measure the implicit valence of drug-associated cues is the affective startle modulation (Geier *et al.*, 2000; Rehme *et al.*, 2009). The acoustic startle reflex (ASR) is a solid marker of emotional reactivity (Lang *et al.*, 1990) and is elicited by unexpected, intense environmental stimuli such as a sudden, loud noise. The affect-modulated

startle methodology has been repeatedly used in drug research by including photographs of drug paraphernalia or drug intake rituals and measuring the suppression of the startle response by drug-related scenes relative to neutral scenes (referred to as cue-related startle suppression, CSS). In general, these studies confirmed incentive theories because they consistently reported CSS in addicted users of alcohol or nicotine while viewing drug-related cues (Cinciripini *et al.*, 2006; Dempsey *et al.*, 2007; Geier *et al.*, 2000; Mucha *et al.*, 2000). Enhanced attentional processing of heroin cues and cue-induced reactivity have been demonstrated for heroin users with other methods, like Stroop tasks and electrodermal responses (Franken *et al.*, 2000; Lubman *et al.*, 2008), and some of these found that cue reactivity might be a predictor for future heroin use and relapse after treatment (Fatseas *et al.*, 2011; Lubman *et al.*, 2009; Marissen *et al.*, 2006). However, the implicit valence of heroin cues before and after detoxification and withdrawal therapy was not investigated so far. Furthermore, it is unknown whether heroin cues elicit appetitive effects in long-term heroin-abstinent individuals. However, sustained appetitive value of drug-related cues might be a driving force underlying the high relapse rates in heroin addiction (Carter and Tiffany, 1999; Fatseas *et al.*, 2011).

Hence, we conducted two studies investigating the implicit valence of drug-related stimuli in different states of abstinence. In Study I, we analyzed the response to heroin-related cues in current heroin-dependent participants before and after a two week detoxification program, as well as in control participants tested within the same time interval. Smoking cues were used because of the common co-use of nicotine in heroin-dependent patients. For the same reason, for the control group only smokers were recruited. ASR was evoked during the presentation of positive, negative, and neutral pictures from the *International Affective Picture System* (IAPS) (Lang *et al.*, 1997) and drug-related cues in order to assess the implicit valence of stimuli. The subjective evaluation of the stimuli was measured by ratings of valence and craving. According to sensitization and addiction memory theories, we hypothesized that heroin cues have appetitive effects in heroin-dependent participants before and after detoxification.

The aim of Study II was to investigate the influence of successful long-term abstinence on affective processing of heroin-associated cues. Because of the significance of drug-related cues in relapse (Carter and Tiffany, 1999; Fatseas *et al.*, 2011), it is important to investigate whether a history of opiate use is associated with persistent implicit memories and enhanced cue-reactivity or if processing changes after successful abstinence. Therefore, a group of long-term abstinent heroin users was compared with current heroin users before detoxification and

controls. Here, we expected long-term abstinent heroin-dependent participants to be more similar to current opiate users than to controls in their implicit reactivity towards heroin cues, as the incentive sensitization theory and addiction memory models posit memory traces to be persistent.

2.3 Experimental procedures

2.3.1 Participants

Study I was carried out with two groups: 15 currently heroin-dependent participants and 15 healthy participants with no history of opiate use (Table 1). Heroin-dependent participants were enrolled in a residential detoxification program at the Department of Psychiatry of the University of Bonn, Germany. Intravenous heroin dependence was diagnosed following the Diagnostic and Statistical Manual-IV (DSM-IV) criteria (American Psychiatric Association, 1994). Detoxification therapy was standardized by treatment with methadone (up to 20mg/day), which was decreased within 8-12 days of inpatient treatment. None of the patients in the detoxification program received methadone maintenance treatment prior to detoxification. The control group was recruited from the general population by advertisement.

Study II compared a non-overlapping sample of 14 long-term abstinent heroin users (assessed once) with the 15 heroin-dependent participants (before detoxification) and 15 control participants (first measurement) from Study I. Long-term abstinent heroin users had to be diagnosed previously with intravenous heroin dependence according to DSM-IV criteria. They were recruited from a study on genetic influences on vulnerability to heroin dependence (Xu *et al.*, 2004). In conjunction with a five-year follow up, only subjects who were abstinent for at least one year were considered for the present study (mean abstinence duration (SD)=18.93 (±20.45) months). They did not receive methadone or heroin maintenance therapy. Abstinence was confirmed by urine toxicology.

All participants were active smokers. Exclusion criteria comprised psychiatric or neurological disorders, and substance use disorders other than nicotine and opioid dependence for heroin users, use of psychotropic medication, and medical conditions concerning eyes, ears and equilibrium organs.

The study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn and was carried out in accordance with the declaration of Helsinki. After receiving a written and oral description of the aim of this study, all participants provided written informed-consent statements and received either 50€ (current heroin users and controls) or 25€ (former heroin users) for their participation.

2.3.2 Experimental procedures

For Study I heroin-dependent participants were first tested when they started detoxification therapy (T1) and again when they finished therapy after 14 days of treatment (T2). Accordingly, control subjects were also tested on two experimental days with an interval of 14 days. In Study II, long-term abstinent heroin users were tested once and compared to heroin-dependent participants and controls at T1. Before the experiment, abstinent heroin users had to provide a urine sample to test for abstinence from opiates and other illegal drugs.

All participants were asked for their drug history, daily cigarette consumption, momentary desire for heroin and momentary withdrawal symptoms by means of self-designed questionnaires. The questionnaire for withdrawal symptoms included 23 symptoms according to DSM-IV criteria. Their occurrence had to be rated on a 5-point scale. Further, the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-A) (Lehrl, 1999), a standardized German vocabulary test, was carried out for the estimation of verbal intelligence quotient (IQ). The Hopkins Symptom Checklist (SCL-90-R) (Franke, 1995) was employed as a self-report psychological status symptom inventory. Before the startle procedure, all participants underwent a hearing screening to insure hearing within normal limits. Participants would have been excluded on the basis of hearing impairment at 40dB (1000Hz). Participants had to abstain from smoking for at least 60min prior to the study, as the acute effects of cigarette smoking have been shown to influence startle amplitudes (Kumari *et al.*, 1996). The startle experiment was carried out in a calm and darkened room. Subjects were instructed to view the pictures and to ignore any noises from the headphones. After the startle procedure, all subjects viewed each picture individually and rated the perceived valence of the picture on 10-point scales (i.e., “How do you perceive the present picture?”, 0=very unpleasant, 10=very pleasant), as well as their craving for nicotine and heroin (i.e., “How much desire to smoke/to take heroin does the present picture induce?”, 0=no desire, 10=strong desire).

2.3.3 Stimulus material and presentation

Stimulus material comprised 56 colored photographs, sixteen pictures showing the beginning and end of a heroin injection scene (**Supplementary Figure S1**), and 16 pictures showing the beginning and end of smoking a cigarette, which were taken from a previous study (Mucha *et al.*, 1999, Experiment 2) (**Supplementary Figure S2**). Drug-associated stimuli depicted the beginning and end scenes of drug use because begin- and end-pictures of smoking and alcohol

consumption scenes were shown to be processed differently (Mucha *et al.*, 2008; Nees *et al.*, 2011; Stippekohl *et al.*, 2010). Twenty-four control pictures comprised eight pleasant, neutral and negative scenes or objects, respectively. The task was presented using the Experimental Run Time System (ERTS) software (Berisoft Cooperation, Frankfurt, Germany). Details on the stimulus material are given in the Supplementary text.

Pictures were arranged in two blocks of 28 photographs in a fixed-randomized and balanced order. Each block started with a 4-min habituation period of 70dB background white noise. The pictures were presented for 7 to 8s, followed by a black monitor lasting for 16.5 to 25.5s. During 6 out of 8 pictures (75%) of each category an acoustic startle response (ASR) was evoked, resulting in 40 ASR trials. The startle probe consisted of a burst of white noise with an intensity of 116dB (duration 40ms, instantaneous rise/fall time, bandwidth approximately 300 Hz-18 kHz) presented binaurally using headphones (TDH-39-P; Maico). Startle probes were presented 2.5, 4.5, and 5.5s after picture onset.

2.3.4 Data recording and reduction

ASR was recorded and analyzed as described previously (Rehme *et al.*, 2009). In brief, the eye-blink component of the ASR was measured by an electromyographic (EMG) startle system (EMG-SR-Lab; San Diego Instruments, Inc., San Diego, CA). EMG activity was measured from the right orbicularis oculi muscle using two silver/silver chloride electrodes. A reference electrode was placed on the glabella. Detailed recording settings and data reduction procedures are given in the **Supplementary material**.

2.3.5 Data analysis

Demographic variables, smoking and heroin using behavior and craving measures were analyzed using analyses of variance (ANOVAs). Differences in sex distribution between groups were tested using χ^2-tests.

The individual startle amplitudes were standardized according to the individual mean and the standard deviation of the startle amplitudes of the control scenes. Standardization was appropriate as the raw startle magnitude showed strong variation across subjects (Patrick *et al.*, 1993). Age was introduced as a covariate because it has been shown to influence affective startle modulation (Feng *et al.*, 2011). Therefore, the standardized startle amplitudes were analyzed parametrically by mixed-design ANCOVAs controlling for age. The difference between

the standardized amplitude during neutral scenes minus the standardized amplitude during drug-related scenes was used as an index for the CSS. Details on the data analyses are given in the **Supplementary material**. ANCOVAs were followed by Bonferroni-corrected pair-wise comparisons and simple main effects analyses. Statistical tests were considered significant at a level of $p<0.05$ (two-tailed). Analyses were performed using the PASW Statistics 18 for Windows.

2.4 Results

2.4.1 Study I: current heroin-dependent participants before and after detoxification therapy

2.4.1.1 Demographic characteristics

Healthy control subjects and heroin-dependent participants did not differ in verbal IQ, age, cigarettes smoked per day (CPD) or sex distribution. Heroin-dependent participants had less years of education (F(1,29)=8.56, *p*=0.007) and, as expected, scored higher on the SCL-90-R sum score (F(1,29)=9.23, *p*=0.005). Sociodemographic characteristics and drug use parameters are presented in **Table 1 and 2**.

Table 1. Demographic characteristics (mean and standard deviation [SD]) of controls, current heroin users and former heroin users. Statistics were carried out for Study I (current heroin users vs. healthy controls) and Study II (current heroin users vs. former heroin users vs. healthy controls).

	Healthy controls (n=15)	Current heroin-dependent (n=15)	Former heroin-dependent (n=14)	**Study I** Current heroin dependent vs. healthy controls			**Study II** Current heroin dependent vs. former heroin dependent vs. healthy controls		
Study	I & II	I & II	II	Value[a]	df/dferr	p	Value[a]	df/dferr	p
Male participants	12	14	9	$\chi2 = 1.154$	1	0.283	$\chi2 = 3.758$	2	0.153
Age	29.53 (6.66)	30.93 (7.09)	35.57 (5.79)	F = 0.311	1/29	0.582	F = 3.339	2/41	0.045*
Years of education	10.87 (2.13)	9.20 (0.56)	10.36 (1.36)	F = 8.562	1/29	0.007*	F = 4.795	2/41	0.013*
Verbal IQ	95.70 (10.97)	92.36 (7.08)	102.00 (5.97)	F = 0.935	1/29	0.348	F = 4.800	2/41	0.014*
SCL-90-R sum score	32.73 (26.11)	61.87 (26.41)	35.14 (26.33)	F = 9.228	1/29	0.005*	F = 5.628	2/41	0.007*
Craving for heroin (0-80)	-	31.07 (11.03)	24.78 (7.51)	-	-	-	F = 3.167	1/28	0.086
Withdrawal symptoms (0-115)	-	41.93 (12.81)	24.79 (7.51)	-	-	-	F = 8.133	1/28	0.008*

[a]ANOVA oder Chi²-test
*Indicates significant differences between groups

Table 2. Duration and quantity of drug use of controls, current heroin users and former heroin users (mean and standard deviation [SD]).

	Healthy controls (n=15)	Current heroin-dependent (n=15)	Former heroin-dependent (n=14)
Study	I & II	I & II	II
Heroin			
Days of use[a]	-	28.00 (4.14)	-
Years of use	-	6.27 (3.77)	8.57 (7.35)
Nicotine			
Cigarettes per day (CPD)	19.60 (8.57)	20.17 (10.87)	16.62 (6.20)
Years of use	10.47 (5.37)	16.40 (3.05)	19.08 (5.68)
Alcohol			
Days of use[a]	6.40 (7.99)	6.80 (9.74)	3.50 (5.95)
Years of use	0.87 (2.64)	3.27 (3.73)	5.64 (6.92)
Benzodiazepines			
Days of use[a]	-	0.93 (1.94)	-
Years of use	-	1.00 (2.48)	0.30 (0.61)
Cocaine			
Days of use[a]	-	3.80 (8.18)	-
Years of use	-	0.67 (1.23)	4.54 (7.26)
Amphetamines			
Days of use[a]	-	0.4 (1.30)	-
Years of use	-	0.53 (1.30)	0.64 (1.34)
Cannabis			
Days of use[a]	2.73 (5.60)	3.13 (7.61)	2.43 (7.98)
Years of use	1.20 (2.81)	5.80 (6.09)	6.50 (8.20)

[a]Days of use refers to the number of day on which the drug was used during the last 30 days.

2.4.1.2 Startle modulation by negative, neutral and positive standardized stimuli

Raw startle magnitudes did not differ significantly between groups on first (T1) and second (T2) assessment (all $p>0.250$), although heroin users showed slightly decreased startle reactivity (**Supplementary Material Figure S3).** Negative, neutral and positive pictures from the IAPS induced a linear startle pattern ($F(1,28)=19.83$, $p<0.001$) in T1 and T2 (**Figure 1A and B**). The mixed-design ANCOVA yielded no group, time or picture category main effects (all $p>0.220$) and no significant interactions (all $p>0.255$). A priori predicted pair-wise comparisons revealed significant differences between negative and positive stimuli and positive and neutral stimuli across groups (all $p<0.001$).

2.4.1.3 Drug cue effects

A mixed-design ANCOVA revealed a significant time*category interaction (F(3,81)=3.17, *p*=0.030) and category*group interaction (F(3,81)=2.73, *p*=0.050). Heroin-dependent participants at T1 showed a significant CSS during heroin end (F(1,28)=5.78, *p*=0.023) and smoking end pictures (F(1,28)=10.10, *p*=0.004). In control participants, a significant CSS was found during smoking end pictures (F(1,28)=12.07, *p*=0.002). A significant difference between T1 and T2 was found for smoking end pictures in heroin-dependent participants (F(1,28)=9.84, *p*=0.004). In control participants, no significant differences were found between T1 and T2 (all *p*>0.268) (**Figure 1C and D**).

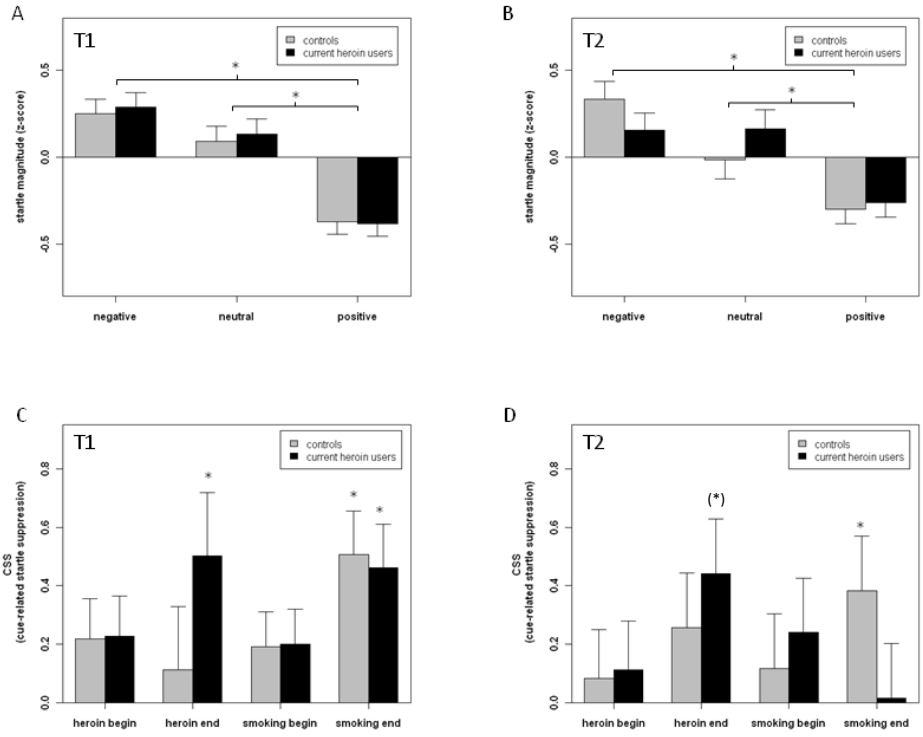

Figure 1. Mean standardized startle amplitudes during the presentation of control pictures on T1 (A) and T2 (B) and mean CSS during the presentation of drug-related cues on T1 (C) and T2 (D). CSS was computed by subtracting the mean standardized startle amplitudes for drug-related pictures from mean standardized startle amplitudes for neutral pictures. Means are adjusted for age. Error bars refer to SEM. *Indicates significant (p<0.05) differences between conditions (A and B) and significant (p<0.05) differences between standardized startle amplitudes in neutral and drug-related scenes (C and D). $^{(*)}$Indicates difference between standardized startle amplitudes in neural drug-related scenes p<0.1)

2.4.1.4 Explicit picture rating (valence)

A significant main effect for picture category on valence ratings was found for the control pictures (F(2,56)=120.94, *p*<0.001.) Both groups rated the IAPS pictures according to their assumed valence (all paired comparisons p<0.001) at T1 and T2 (data not shown).

For drug-associated pictures, a significant main effect for category (F(3,81)=34.12, *p*<0.001) and significant interactions for category*group (F(3,81)=5.90, *p*=0.001) and time*group (F(3,81)=4.94, *p*=0.035) were found. At T1 and T2, heroin-dependent participants rated heroin begin and end cues significantly more positive than control participants (all *p*<0.010). No significant group difference was found for smoking pictures (all *p*>0.081).

Furthermore, heroin-dependent participants rated heroin end pictures (F(1,28)=5.13, *p*=0.032) and smoking begin pictures (F(1,28)=11.68, *p*=0.002) significantly more pleasant after therapy than before, whereas control participants showed no change in their valence ratings between T1 and T2 (all *p*>0.468) (**Figure 2A and B**).

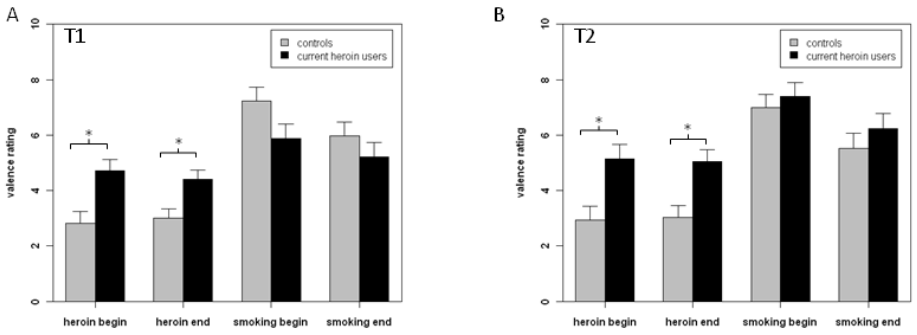

Figure 2. Mean valence ratings during drug-related pictures onT1 (A) and T2 (B) for controls (n=15) and current heroin users (n=15). Means are adjusted for age. Error bars refer to SEM. *Indicates significant (p<0.05) differences between groups.

2.4.1.5 Explicit picture rating (craving)

With regard to the ratings of craving for heroin, a significant main effect for group (F(1,25)=18.54, *p*<0.001) and category (F(3,75)=15.84, *p*<0.001) and a significant interaction for category*group (F(3,75)=15.91, *p*<0.001) were found. At T1, heroin-dependent participants reported more craving than controls for heroin begin (F(1,25)=11.410, *p*=0.002) and heroin end pictures (F(1,25)=13.53, *p*=0.001), while no significant difference was found for smoking cues. There was no significant difference in craving ratings between T1 and T2 (all *p*>0.093). At T2, heroin-dependent participants reported more craving for heroin than control subjects

during all drug-related picture categories (heroin begin: F(1,25)=12.82, *p*=0.002; heroin end: F(1,25)=11.50, *p*=0.002; smoking begin: F(1,25)=4.23, *p*=0.050; smoking end: F(1,25)=4.91, *p*=0.036) (**Figure 3A and B**). Craving to smoke cigarettes differed neither between groups nor time points (**Supplementary Material Figure S4A and S4B**).

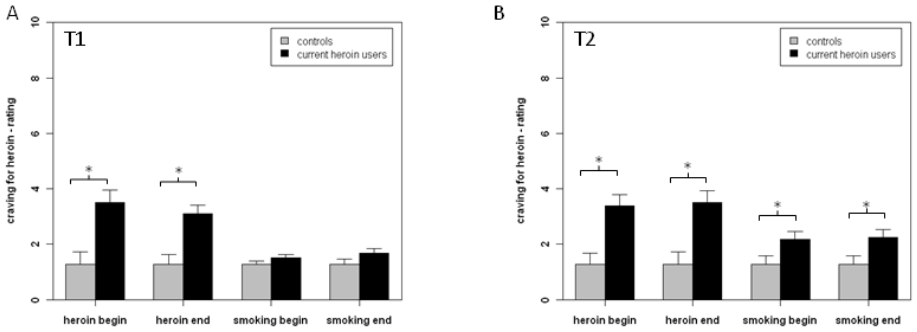

Figure 3. Mean craving for heroin-ratings during drug-related pictures on T1 (A) and T2 (B) for controls (n=15) and current heroin users (n=15). Means are adjusted for age. Error bars refer to SEM. *Indicates significant (p<0.05) differences between groups.

2.4.2 Study II: Long-term abstinent heroin users

2.4.2.1 Demographic characteristics

Abstinent heroin users, heroin-dependent participants and control participants showed a comparable sex distribution ($\chi^2_{(2)}$=3.75, p=0.153) and did not differ in CPD (F(2,42)=3.68, p=0.537). However, groups differed in age, years of education, IQ, and SCL-90-R sum score (all p<0.045). Abstinent heroin users and heroin-dependent participants differed in the heroin withdrawal score (F(1,28)=8.13, p=0.008) (**Tables 1 and 2)**.

2.4.2.2 Startle modulation

For IAPS control pictures, the mixed-design ANCOVA yielded neither a significant main effect nor an interaction (all p>0.074). As a priori predicted, pair-wise comparisons revealed significant startle magnitude differences between negative and neutral stimuli (p<0.001) as well as between negative and positive pictures (p<0.001) (**Figure 4A**).

Comparing startle magnitudes during drug-related cues between controls and abstinent heroin users, a 4*2 (category*group) mixed-design ANCOVA showed a significant main effect for group (F(1,26)=5.45, p=0.028). There was no significant difference between abstinent heroin users and current heroin-dependent participants (F(1,26)=0.68, p=0.419). Former heroin users showed a significant CSS for heroin begin and heroin end pictures (all p<0.050) (**Figure 4B**). Raw startle magnitude was slightly smaller in current heroin users, but did not differ significantly between groups (F(2,43)=1.12, p=0.335) (**Supplementary Material Figure S5**).

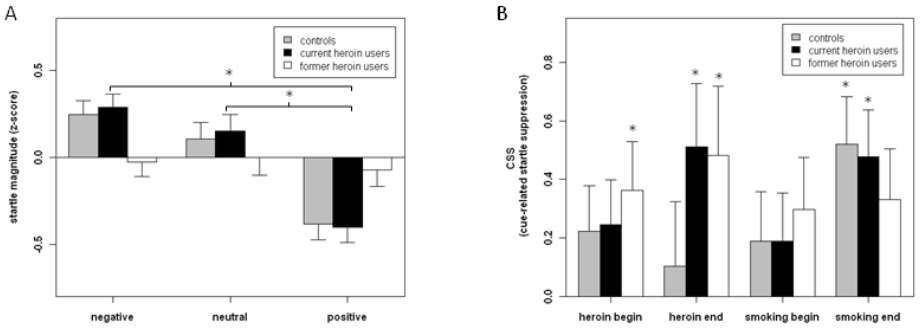

Figure 4. Mean standardized startle amplitudes during the presentation of control pictures (A) and mean CSS during the presentation of drug-related cues (B) for controls (n=15), current heroin users (n=15) and former heroin users (n=14). CSS was computed by subtracting the mean standardized startle amplitudes for drug-related pictures from mean standardized startle amplitudes for neutral pictures. Means are adjusted for age. Error bars refer to SEM. Controls and current heroin users in Study II are the same as in Study I. *Indicates significant (p<0.05) differences between conditions (A) and significant (p<0.05) differences between standardized startle amplitudes in neutral and drug-related scenes (B).

2.4.2.3 Explicit picture rating (valence)

For the IAPS pictures, there was a significant main effect of picture category (F(2,82)=66.13, p<0.001). The pictures were rated by all three groups according to their assumed valence (all paired comparisons p<0.001) (data not shown).

For drug-associated pictures, there was a significant main effect for picture category (F(3,123)=43.50, p<0.001) and a significant interaction of category*group (F(6,123)=6.53, p<0.001). Abstinent heroin users rated heroin begin (p=0.022) and heroin end (p=0.028) significantly less pleasant than current heroin-dependent participants. No difference could be

found between abstinent heroin users and controls in their valence rating of heroin-associated pictures (all p>0.427). Ratings for smoking pictures were not different between heroin-dependent participants and abstinent heroin users (all p>0.705). Abstinent heroin users rated smoking begin cues (p=0.050) and smoking end pictures (p=0.033) significantly less pleasant than control participants (**Figure 5A**).

2.4.2.4 Explicit picture rating (craving)

The analysis of ratings of craving for heroin revealed a significant main effect for group ($F(2,38)=10.21$, $p<0.001$) and category ($F(3,114)=12.38$, $p<0.001$) and a significant interaction of category*group ($F(6,114)=7.26$, $p<0.001$). For heroin begin and end pictures, abstinent heroin users showed significantly less craving than current heroin-dependent participants (p=0.014, p=0.004). There was no difference in craving between controls and abstinent heroin users (all p>0.147). For smoking pictures, no group differences were found (all p>0.170) (**Figure 5B**). Ratings of craving to smoke showed no significant group differences (**Supplementary Material Figure S6**).

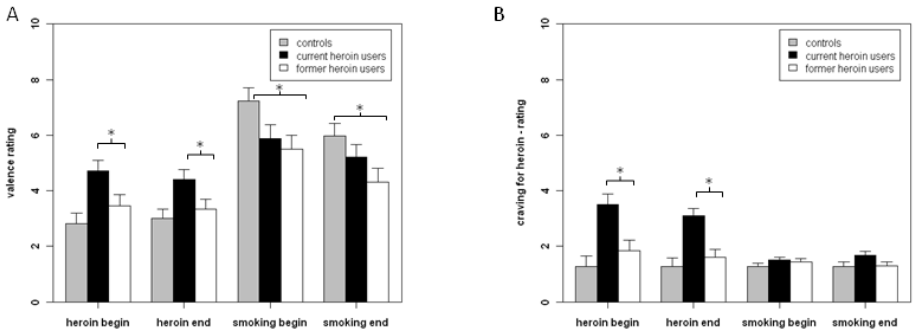

Figure 5. Mean valence (A) and craving for heroin (B) ratings during drug-related pictures for controls (n=15), current heroin users (n=15), and former heroin users (n=14). Means are adjusted for age. Error bars refer to SEM. Controls and current heroin users in Study II are the same as in Study I. *Indicates significant (p<0.05) differences between groups.

2.5 Discussion

The present study assesses the implicit and explicit reactivity to heroin-related cues before and after detoxification therapy and after long-term heroin abstinence. Heroin-dependent patients show normally modulated affective reactivity to emotional control pictures suggesting that the overall emotional reactivity is not impaired, which is in line with Walter *et al.* (2011), who also failed to find differences in startle reactivity to emotional pictures. However, results are inconsistent with Lubman *et al.* (2008; 2009) reporting reduced responsiveness to natural reinforcers in heroin users previously. Heroin-dependent participants, in contrast to controls, show a significant reduction in startle response during stimuli depicting the end of a heroin injection scene compared to neutral stimuli. Valence ratings confirm that heroin users perceive heroin pictures as more pleasant than control participants, whereas both groups show no difference in valence ratings of smoking pictures.

This is in line with incentive theories of addiction proposing that drug associated cues are processed as appetitive or motivational incentives (Robinson and Berridge, 2000; Stewart, 1983; Wise, 1988). Incentive theories of drug addiction have been confirmed for other drugs, e.g., nicotine (Dempsey *et al.*, 2007; Geier *et al.*, 2000; Rehme *et al.*, 2009) and alcohol (Grüsser *et al.*, 2002; Heinz *et al.*, 2003; Mucha *et al.*, 2000), but results for heroin cues were undetermined so far. Walter *et al.* (2011) did not find a difference in startle response between neutral and drug-related stimuli, but opposed to our study, they used less specific and standardized drug pictures. Moreover, their patients were recruited from an opioid-maintained treatment program and were treated with stable doses of methadone or heroin. Thus, opioid-maintanance might damp the emotional reactivity of heroin-dependent patients (Walter *et al.*, 2011). Our finding that heroin-cues are not perceived as aversive but serve as emotional and maybe motivational incentives is also in line with recent findings suggesting enhanced activation of reward-related brain areas (e.g., the anterior cingulate cortex and basal ganglia) in response to heroin-related cues in heroin users (Wang *et al.*, 2011).

One novel finding is that the incentive value of heroin-related cues for heroin users persists after a 14-day detoxification therapy. Interestingly, explicit valence ratings imply that heroin end and smoking begin pictures are experienced as more pleasant after detoxification than before. These results are consistent with the finding that alcohol-related stimuli have appetitive qualities during alcohol detoxification and early abstinence (Grüsser *et al.*, 2002). In addition, it has been shown that the reward system remains responsive to heroin-related cues

in methadone maintenance patients with a history of heroin use (Langleben *et al.*, 2008) and recent abstinence (Daglish *et al.*, 2001). Nevertheless, craving in response to heroin-related cues did not change after detoxification therapy. This finding is in line with previous studies proposing a protracted abstinence syndrome even after longer drug free periods (Fatseas *et al.*, 2011; Shi *et al.*, 2007). Thus, strong appetitive effects of drug-related cues may support relapses after successful detoxification.

Another novel finding of the present study is that implicit incentive effects can still be measured even after at least one year of abstinence. Former heroin users do not differ from current heroin users, both groups showing suppressed startle responses to heroin-related stimuli. Recently, Shi *et al.* (2008) found a persistent decrease in the brain dopamine transporter (DAT) in the striatum of prolonged abstinent heroin abusers. Together, these findings support theories of an addiction memory, possibly based on long-lasting, or even permanent alterations in the dopamine system, which increases the vulnerability to relapse even after long-term abstinence (Kalivas and Volkow, 2005; Kelley, 2004). In sum, these findings corroborate the close association between reward-related learning, memory, and addiction postulated by theories of addiction and addiction memory (Berke and Hyman, 2000; Hyman and Malenka, 2001; Hyman *et al.*, 2006; Kelley, 2004; Robbins and Everitt, 2002; White, 1996).

Notably, the implicit appetitive reactions of former heroin users to heroin-related stimuli are in contrast with their explicit measures: Valence and craving ratings of heroin-related stimuli did not differ between former heroin users and controls. This finding supports the hypothesis that explicit and implicit processing of drug-related cues might be dissimilar (Grüsser *et al.*, 2002; Tiffany, 1990). Even though sensitization of neural pathways seems to be persistent, former heroin users are obviously able to maintain abstinence. Top-down controlled processes might be supportive to inhibit drug-cue driven approach behaviors. Cognitive strategies of devaluating drug-related cues have been shown to enhance abstinence in smokers (Rose, 2006). Recently, Min *et al.* (2011) reported higher abstinence rates when heroin users underwent a relapse prevention program after detoxification. The training of cognitive strategies to reduce the motivational impact of heroin-related cues should therefore be considered as a crucial part of relapse prevention programs.

The present study has some limitations. First, the sample comprised mainly men, who differ from women in their subjective and physiological reactions to heroin-related cues as it has been previously shown (Yu *et al.*, 2007). The results should therefore be replicated in women to be generalized. Second, even though urine toxicology confirmed current abstinence in

former heroin users, abstinence duration measures rely on self-report and cannot be verified objectively, which is, however, an inevitable constraint. Third, in Study I, methadone treatment has been reduced two days before T2, which might have influenced emotional reactivity. However, Savvas *et al.* (2012), showed that effects of methadone on emotional processing were most prominent at peak plasma concentrations (around 3h post-dose) but 24h later no differences in emotional reactivity were found between controls and opioid-dependent subjects. Thus, we considered the residual influence of methadone on emotional reactivity to be very low at T2. Fourth, the sample size is moderate. However, this study (Study I) is the first longitudinal study employing an electrophysiological approach to measure the incentive value of drug-related cues. Moreover, it is also the first study comparing current and long-term abstinent heroin users with controls (Study II). Thus, despite of the moderate sample size this study provides unique insights regarding the stability of incentive values of heroin-related cues in heroin users. Finally, in Study I, it cannot be ruled out that test-retest effects influenced the results of the second assessment, although the startle reaction is involuntary and assessments were separated by two weeks.

2.6 Conclusions

Heroin-related cues showing the end of a heroin injection scene are appetitive in current heroin-dependent subjects, and these cues retain their appetitive valence even after a detoxification therapy of 14 days as well as after long-term abstinence for more than one year. These findings indicate that chronic heroin use leads to stable and long-lasting adaptations of neural pathways resulting in a high risk for relapse over a long time period. However, cognitive control processes seem to play a crucial role to maintain abstinence despite increased reactivity to heroin-related cues because explicit and implicit reactions are dissociated in long-term abstinent heroin users.

Further studies are needed to examine whether the implicit appetitive response to heroin-related cues declines after prolonged abstinence for more than one year and whether this can be influenced by special treatment programs. Further, interventions which strengthen the devaluation of drug cues and apply stimulus control techniques should be part of relapse prevention programs.

2.7 Role of the funding source

Experimental design, data acquisition, statistical analyses, statistical analysis, and interpretation of the results were conducted without input from any pharmaceutical company or any other funding source.

2.8 Conflict of interest

All authors report no biomedical financial interests or potential conflicts of interest with respect to this study.

2.9 Acknowledgments

Katrin Preller and Dr. Boris B. Quednow were supported by grants from the Swiss National Science Foundation (SNSF; grant No. PP00P1_123516). The authors are grateful to Dr. Ron Mucha for providing stimulus material for the present study.

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2.11 Supplementary text

Data recording and reduction

The eye-blink component of the acoustic startle response was measured by an electromyographic (EMG) startle system (EMG-SR-Lab; San Diego Instruments, Inc., San Diego, CA). EMG activity was measured from the right *orbicularis oculi* muscle using two silver/silver chloride electrodes. A reference electrode was placed on the glabella. All electrode resistances were less than 10kΩ. EMG was recorded at a sampling rate of 1000Hz with a notch filter of 50Hz and a band-pass filter between 1 and 1000Hz from the onset of the acoustic startle stimulus for 250 ms.

Voluntary and spontaneous eye blinks were excluded from further analysis using the registration parameters described by Braff *et al.* (1992). The latency to startle response onset was defined by a shift of 2.28µV (six digital units) from the baseline value and occurring in a time window of 21–120ms after the acoustic startle stimulus. Response rejections were made both in case of onset-to-peak latencies >95ms and baseline shifts >34.2µV (>90 digital units). Additionally, startle responses were discarded if the amplitude was more than three standard deviations above the individual mean or if the amplitude was less than 25 digital units. The subject was taken out if there were less than two values per picture category after application of these criteria. Because the amplitude of the startle response underlies a habituation effect over trials (Bradley *et al.*, 1993), we computed a regression analysis in order to correct our data against this effect. The decline of startle magnitude was best described by a logarithmic trend. Consequently, the raw data for every subject in the different trials were corrected for the logarithmic habituation trend. Finally, the available responses for the different picture categories were averaged to obtain the actual score.

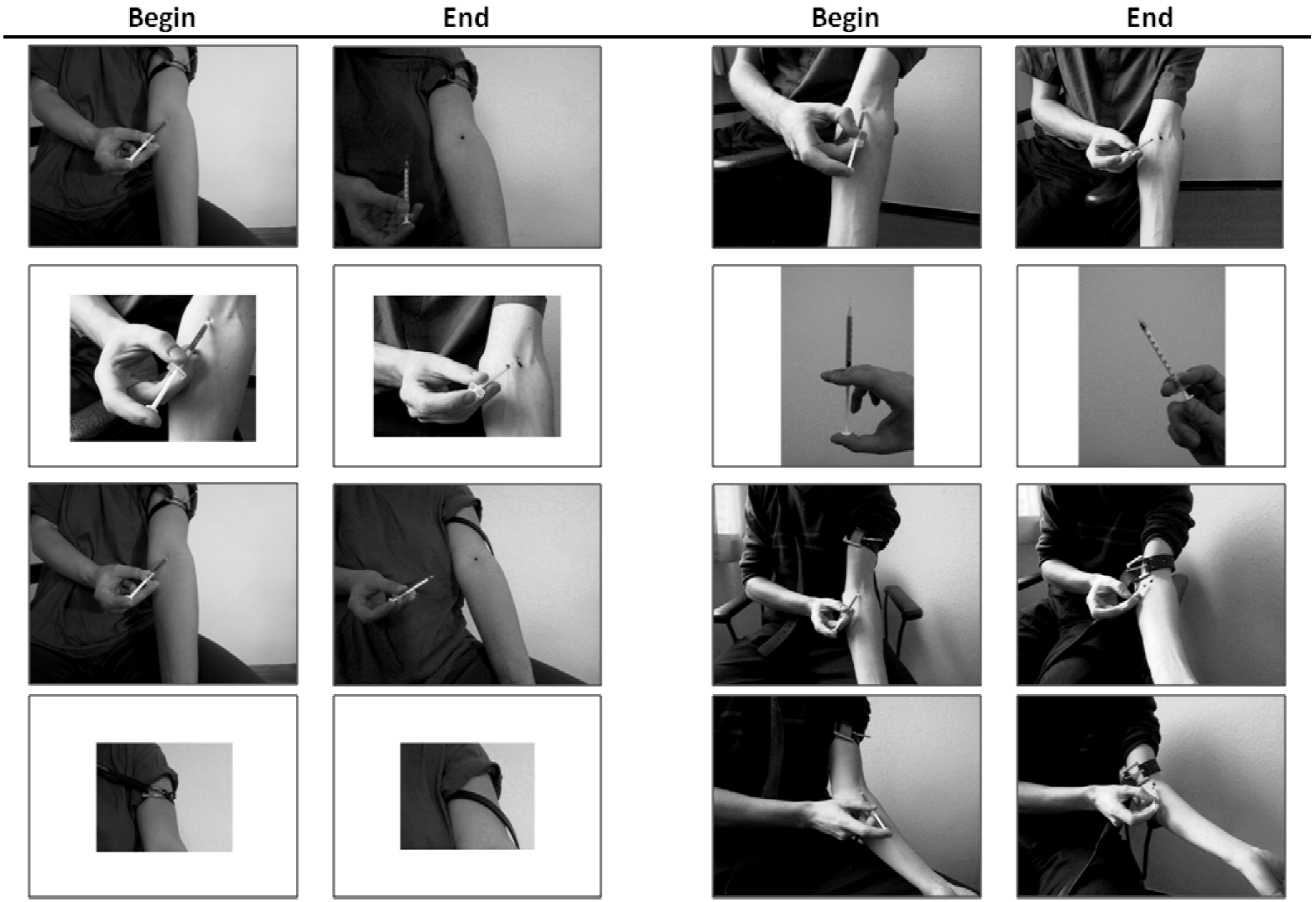

Figure S1. Heroin stimuli used in the affective startle paradigm. The sixteen self-taken pictures were produced as eight pairs showing the beginning and end of a heroin injection scene. During the paradigm they were presented together with neutral, positive, negative and smoking stimuli in a fixed randomized order.

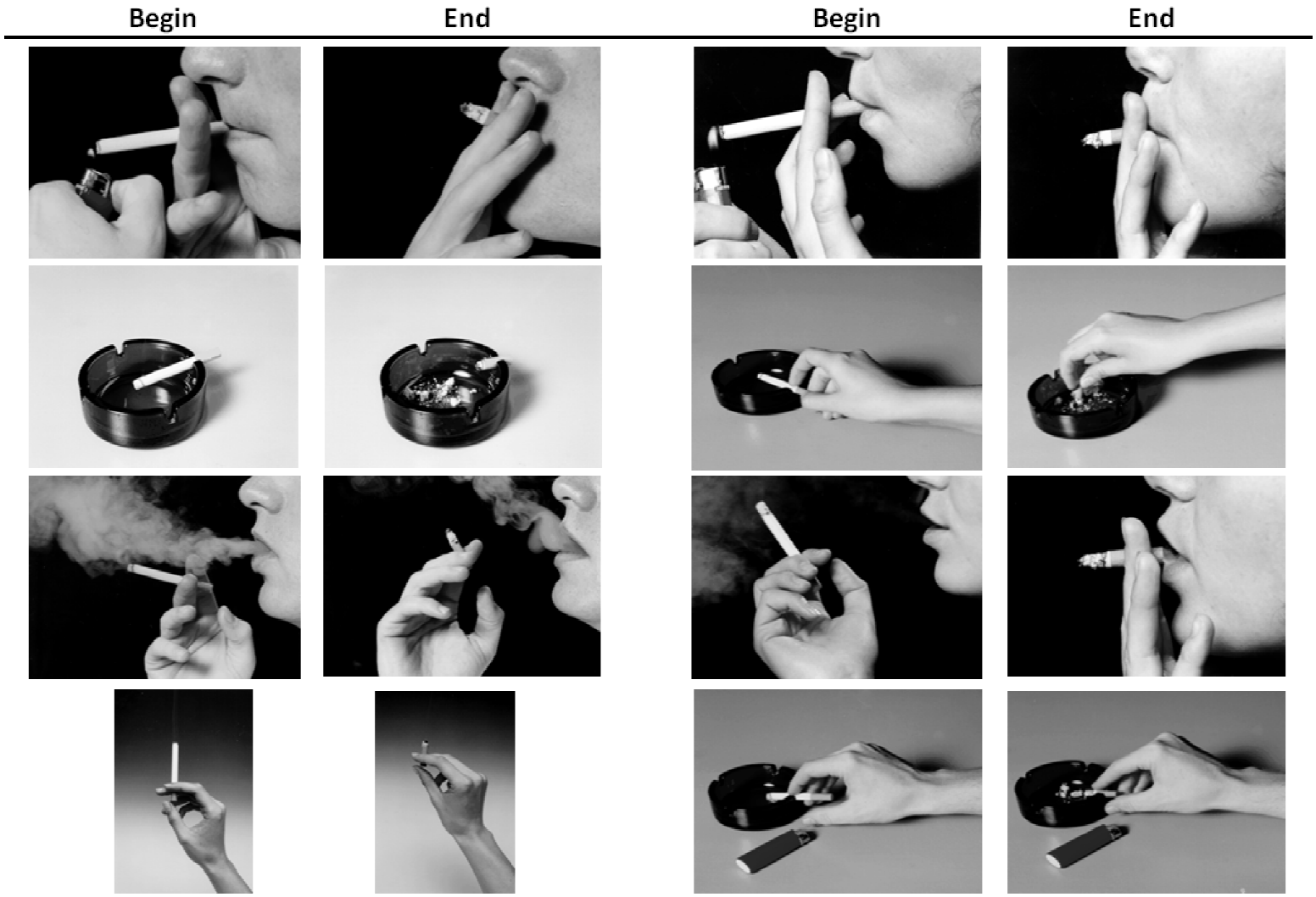

Figure S2. Smoking stimuli used in the affective startle paradigm. The sixteen self-taken pictures were produced as eight pairs showing the beginning and end of a smoking scene. Pictures were taken from a previous study (Mucha *et al.*, 1999, Experiment 2). During the paradigm they were presented together with neutral, positive, negative and heroin stimuli in a fixed randomized order.

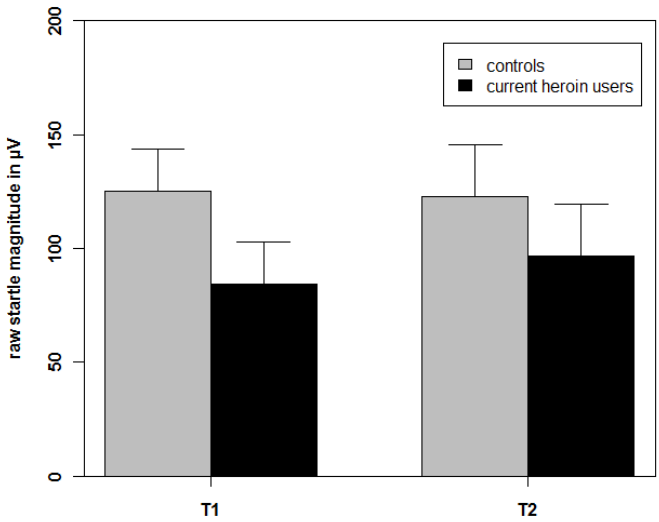

Figure S3. Mean raw startle magnitudes in µV in controls (n=15) and current heroin users (n=15) at T1 and T2. Error bars refer to SEM.

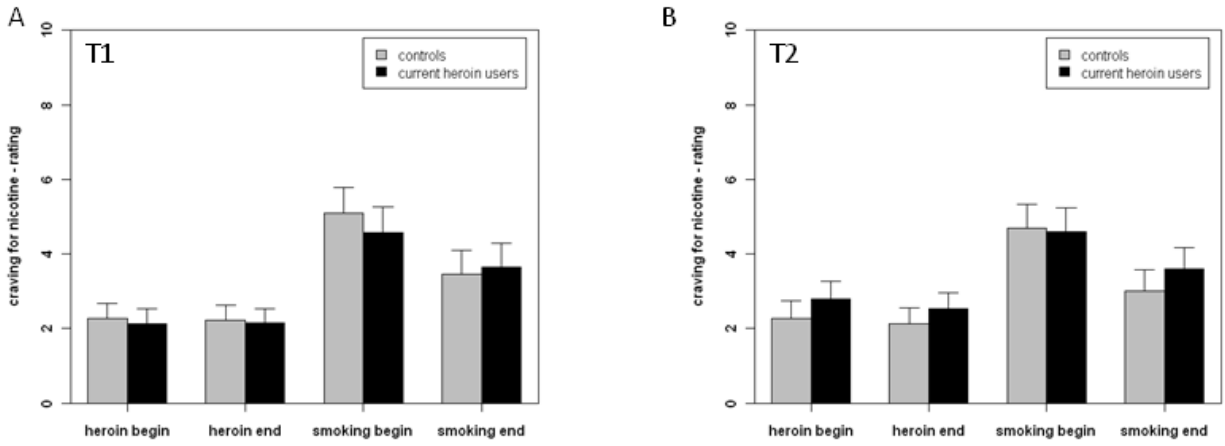

Figure S4. Mean craving for nicotine ratings during drug-related pictures on T1 (A) and T2 (B) for controls (n=15) and current heroin users (n=15). Means are adjusted for age. Error bars refer to SEM.

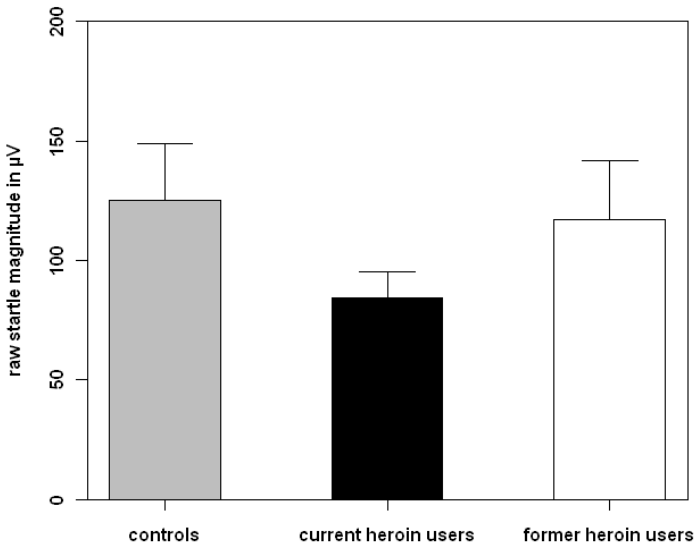

Figure S5. Mean raw startle magnitudes in µV for controls (n=15), current heroin users (n=15) and former heroin users (n=14). Error bars refer to SEM.

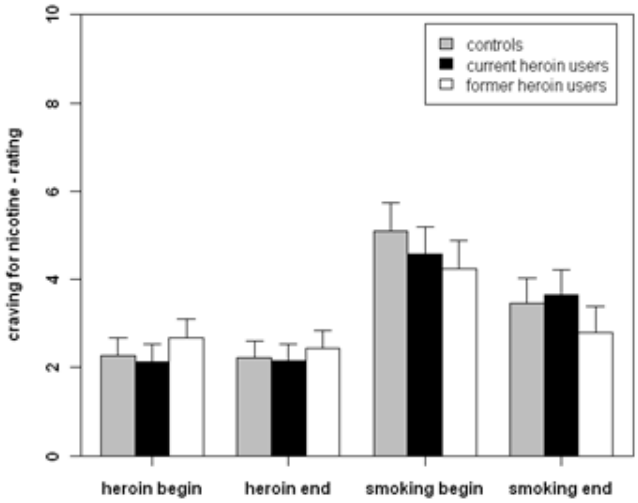

Figure S6. Mean craving for nicotine ratings during drug-related pictures for controls (n=15), current heroin users (n=15), and former heroin users (n=14). Means are adjusted for age. Error bars refer to SEM.

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3

Increased sensorimotor gating in recreational and dependent cocaine users is modulated by craving and ADHD symptoms

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Personal contribution

KHP gathered and analyzed the data, interpreted the data and wrote the manuscript. BBQ designed the study, helped to interpret the data and revised the first draft of the manuscript. NI contributed to data acquisition, data analysis and data interpretation. LMH, MV, DJ contributed to data acquisition and/or revised the first draft of the manuscript. MRB conducted the hair analyses. FXV revised the first draft of the manuscript.

3.1 Abstract

Background

Cocaine dependence has been associated with blunted dopamine and norepinephrine signaling, but it is unknown if recreational cocaine use is also associated with alterations of catecholamine systems. Prepulse inhibition (PPI) of the acoustic startle response (ASR) – a measure of sensorimotor gating – is highly sensitive for manipulations of the catecholamine system. Therefore, we investigated whether relatively pure recreational (RCU) and dependent cocaine users (DCU) display alterations of PPI, startle reactivity, and habituation. Moreover, the influences of methylenedioxy-methamphetamine and cannabis co-use, craving, and attention-deficit/hyperactivity Disorder (ADHD) symptoms on startle measures were examined.

Methods

In 64 RCU, 29 DCU, and 66 stimulant-naïve control subjects, PPI of ASR, startle reactivity, habituation, ADHD symptoms, and cocaine craving were assessed. Drug use of all participants was controlled by hair and urine toxicologies.

Results

Both, RCU and DCU showed increased PPI in comparison with control participants (Cohen's *d*=.38, *d*=.67, respectively), while RCU and DCU did not differ in PPI measures (*d*=.12). No significant group differences were found in startle reactivity or habituation measures. In cocaine users, PPI was positively correlated with cumulative cocaine dose used, craving for cocaine, and ADHD symptoms. Users with a diagnosis of ADHD and strong craving symptoms displayed the highest PPI levels compared with control subjects (*d*=.78).

Conclusion

The augmented PPI in RCU and DCU suggests that recreational use of cocaine is associated with altered catecholamine signaling, in particular if ADHD or craving symptoms are present. Finally, ADHD might be a critical risk factor for cocaine-induced changes of the catecholamine system.

3.2 Introduction

Cocaine is an illegal drug with a high tendency to induce dependence when chronically abused (1). Nevertheless, a substantial part of people use cocaine in a recreational and non-dependent manner (2). The lifetime prevalence of cocaine use is estimated at 5.9% amongst 15- to 34-year olds in Europe. It has been established as the most commonly stimulant drug used in Europe (2) and is the primary stimulant drug responsible for drug-dependence treatment in North and South America (3).

Cocaine inhibits the reuptake of dopamine (DA), norepinephrine (NE), and serotonin (4). Marked structural and functional alterations in striatal and prefrontal regions have been reported in dependent cocaine users (DCU) (5-9). Furthermore, reduced striatal DA D2 receptor availability and blunted striatal DA release have repeatedly been shown to be associated with chronic cocaine use in humans using positron emission tomography imaging (10-13). Striatal DA is a central mediator of reward, memory, and behavioral inhibition (14). Thus, dysregulated DA functioning in DCU has been associated with widespread consequences including craving, impulsive behavior, loss of control over drug intake, and relapse (12,13,15). Recently, upregulation of thalamic NE transporters also has been reported in DCU (16) and NE seems to play an important role in craving, withdrawal-related anxiety, and relapse in cocaine addiction (17,18). While most studies investigate dependent users, little is known about the effects of occasional and recreational cocaine use. However, a recent study showing blue-yellow color vision deficits in recreational cocaine users (RCU) and DCU suggests that recreational cocaine use might lead to changes in DA function (19).

Prepulse inhibition (PPI) of the acoustic startle response (ASR) refers to the attenuation of the reflexive startle reaction when the startling stimulus is preceded 30 milliseconds to 500 milliseconds by a weak and non-startling stimulus (20). PPI is considered as a translational measure of sensorimotor gating, reflecting a universal preattentional filter function (21) that is regulated by a cortico-striato-pallido-pontine (CSPP) circuit involving the prefrontal cortex, the ventral striatum including nucleus accumbens, the ventral pallidum, and the pontine tegmentum (22). PPI has been shown to be highly sensitive to changes in catecholamine neurotransmission, especially in the ventral part of the mesostriatal DA system (23,24), and in thalamocortical and ventral forebrain NE networks (25-28).

Acute administration of cocaine reduced PPI in rats (29), but lasting effects of repeated drug exposure are less well studied. In cocaine-withdrawn rats, no alterations in PPI were found (29,30). In humans, Efferen *et al.* (31) reported a trend towards increased PPI in a small sample of DCU (n=10 vs. n=9 control subjects). In this study, DCU displayed reduced startle reactivity (31), while a subsequent study revealed that decreased startle was only present in DCU with continued abstinence (>40 days) (32). With regard to startle reactivity, animal results are inconsistent. Adams *et al.* (30) showed reduced startle reactivity in cocaine-withdrawn rats, whereas Martinez *et al.* (29) found no significant differences in startle reactivity or habituation. However, PPI has not been studied in a sufficiently large sample of cocaine users so far. Moreover, the effects of recreational cocaine use on PPI and startle reactivity are unknown. The overlap of reward circuits shown to be altered in cocaine users (in particular the ventral striatum) and CSPP circuits regulating PPI suggests that PPI may be altered in cocaine addiction (9,10,22,23).

Therefore, we aimed to investigate startle reactivity and PPI in large groups of RCU and DCU, and stimulant-naïve control subjects. Preliminary data from a previous study predict increased PPI levels and decreased ASR in DCU (31), while we expected to find a similar but less pronounced pattern already in RCU (19). Given that attention-deficit/hyperactivity disorder (ADHD) is highly comorbid with cocaine dependence and abuse (33) and it was shown that ADHD patients also display alterations in DA and NE signaling (34,35), we additionally assessed the severity of ADHD symptoms in our subjects. Moreover, we examined the severity of cocaine craving, as craving was associated with low striatal DA levels and NE alterations as well (12,36). Psychiatric comorbidities, such as ADHD, and polytoxic drug use have been shown to be confounded in studies especially with DCU (37,38). We aimed to overcome these previous limitations by I) the inclusion of a group of recreational and presumably less comorbid and polytoxic users, II) controlling ADHD symptoms, and III) the application of comprehensive psychiatric diagnostics and the examination of hair toxicologies, allowing the exclusion of subjects with psychiatric diseases (other than substance dependence or ADHD) and polytoxic drug use patterns.

3.3 Methods

3.3.1 Participants

Twenty-nine DCU, 64 RCU, and 66 drug-naïve control participants took part in the study (for recruitment and selection details see **Supplement 1**). Cocaine dependence was diagnosed following the DSM-IV criteria (39), with DCU fulfilling these criteria and RCU not meeting dependency criteria. Further inclusion criteria for the two user groups were cocaine use of at least 1g per month, cocaine as primary used illegal drug, and a current abstinence duration <6 months. Participants had to be aged between 18 and 60 years. Exclusion criteria for the user groups were use of opioids, a polytoxic drug use pattern, and an Axis-I DSM-IV adult psychiatric disorder with exception of cocaine and alcohol abuse/dependence, a history of a depression (acute major depression was excluded), and ADHD. Control subjects were excluded when they displayed any Axis-I DSM-IV psychiatric disorder, inclusive of ADHD, and any form of addiction or regular illegal drug use (lifetime use <15 occasions), with exception of cannabis. Exclusion criteria for all participants were a neurological disorder or head injury, clinically relevant medical diseases, family history of schizophrenia or bipolar disorder, or prescription drugs affecting the central nervous system, and medical conditions concerning eyes, ears, and equilibrium organs. All participants had to abstain from illegal substances for a minimum of three days and from alcohol for at least 24h. Self-reports were controlled by urine and 6-month hair analysis (for details, see **Supplement 1**).

The study was approved by the Cantonal Ethics Committee of Zurich. All participants provided written informed-consent and were compensated for their participation.

3.3.2 Procedure

The present data were collected as part of a larger longitudinal study on socio-cognitive consequences of cocaine use – the Zurich Cocaine Cognition Study ($ZuCo^2St$). A Structured Clinical Interview for DSM-IV Axis I Disorders was carried out by a trained psychologist. Drug use was assessed by means of the Interview for Psychotropic Drug Consumption (40). The brief version of the Cocaine Craving Questionnaire (CCQ) (41) was applied to assess current cocaine craving. The Fagerström Test of Nicotine Dependence (FTND) (42) was used to assess the level of nicotine dependency. The Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) (43), a

standardized German vocabulary test, was carried out for the estimation of premorbid verbal IQ. The Beck Depression Inventory (BDI) (44) measured the current severity of depressive symptoms, and the ADHD self-rating scale (ADHD-SR) (45) was applied to allow for the diagnosis of ADHD in adulthood according to DSM-IV criteria. A neuropsychological test battery was assessed (data will be published elsewhere) and subsequently the ASR measurement was conducted. Participants had to abstain from smoking for at least 60 minutes prior to startle testing.

3.3.3 Startle response measurement

For a detailed description of the paradigm, see **Supplement 1**. In brief, startle stimuli comprised of noise bursts at an 115dB sound pressure level with duration of 40 milliseconds, separated by variable inter-trial-intervals (range: 9-14 seconds, mean: 12 seconds). After an initial pulse-alone trial (PA) the session included a total of 64 trials (56 active and 8 no-stimulation trials). Thirty-two pulse trials were preceded by a 20-msec prepulse with an intensity of 86dB and a stimulus onset asynchrony (SOA) of 30, 60, 120, and 240msec, resulting in four SOA conditions. The eye-blink component of the ASR was measured by an EMG startle system (EMG-SR-Lab; San Diego Instruments, San Diego, California) as described previously (21). Preprocessing of the recorded data was performed using Analyzer software (Brainvision; Brain Products GmbH, München, Germany) and emgBLINK version 1.2 (CST, Zurich, Switzerland) as described in detail previously (46).

3.3.4 Statistical analysis

Frequency data were analyzed by means of Pearson's Chi-square test and quantitative data by analyses of variance (ANOVA) using PASW 18.0 (IBM, Chicago, Illinois). PPI and habituation data were normally distributed in each group (Kolmogorov- Smirnov test $p>.05$) and were therefore analyzed parametrically. The mean %PPI was calculated for each SOA as described previously (21,47). These data were analyzed using mixed-design analyses of covariance (ANCOVA) with Greenhouse-Geisser corrections, followed by Sidak-corrected pairwise comparisons and simple main effects analyses. SOA condition was introduced as within-subjects factor and group as between-subjects factor. As smoking status, sex (3-fold: women differentiated by luteal vs. follicular phase of the menstrual cycle), and age were shown to influence startle parameters (48-52), these variables were introduced as covariates.

Startle reactivity measures were analyzed likewise. To assess habituation, PA trials of block 1 through 4 were analyzed conducting a mixed-design ANCOVA (53). Percent habituation was calculated as the reduction in startle magnitude between the second block and following blocks of PA trials [%Habituation=100*(block 2–block n)/block 2] to avoid sensitization effects (27). Furthermore, the linear gradient coefficient *b* was calculated across the four blocks of PA as described previously (47). %Habituation and *b* were analyzed by one-way ANCOVAs controlling for smoking status, sex, and age.

Correlation analyses (Pearson's product-moment) were conducted to relate drug use parameters to PPI measures. Cumulated cocaine lifetime use was natural logarithm-transformed for statistical analyses because of the highly skewed distribution and the resulting deviation from the normal distribution (Shapiro-Wilk $W<.001$ for both user groups). For correlations between illegal drug use and PPI control subjects were excluded to prevent inflating existing correlations. The confirmatory statistical comparisons of all data were carried out on a significance level set at $p<.05$ (two-tailed).

3.4 Results

3.4.1 Demographic characteristics

The groups did not differ with respect to IQ, sex distribution, and proportion of smokers and non-smokers (**Table 1**). DCU were older than controls and RCU (p<.05) and had fewer years of education than controls (p<.05). As expected, both user groups scored higher on the BDI and ADHD-SR sum scores compared with controls (all p<.01). FTND sum score differed between the smokers in each group (p<.05-.01). The hair samples revealed a clear dominance of cocaine compared with other illegal drugs as strived for by the inclusion criteria (**Table 2)**. DCU showed a more than 7-fold higher concentration of cocaine and metabolites in the hair samples compared with RCU. However, RCU are regular users with a mean weekly consumption of about 1g cocaine but without fulfilling DSM-IV criteria for cocaine dependence. A considerable amount of participants tested positive in urine toxicologies for cocaine and cannabis and we decided not to exclude them but to investigate the acute and post-acute effects of the drugs.

Table 1. Demographic Data (Means and Standard Deviation)

	Control Group (n=66)	Recreational cocaine users (RCU, n=64)	Dependent cocaine users (DCU, n=29)	Value	df/df_{err}	p
Male/ female follicular/female luteal phase (n)	46/15/5	46/9/9	25/3/1	$\chi2$ =5.94[a]	4	0.20
Age (years)	31.11 (9.40)	28.11 (6.24)	36.28 (11.45)[b,c]	F = 8.80[d]	2/156	<0.001[h]
Years of education	10.67 (1.87)	10.42 (1.91)	9.57 (1.24)[b]	F = 3.89[d]	2/156	0.02[h]
Verbal IQ	106.09 (11.20)	102.44 (9.00)	102.10 (10.97)	F = 2.57[d]	2/156	0.08
Smoker/Nonsmoker (n)	50/16	51/13	23/6	$\chi2$ = 0.33[a]	2	0.85
FTND sum score (0-10)[e]	2.24 (2.11)	3.29 (2.10)[b]	4.87 (2.53)[c,f]	F = 11.56[d]	2/122	<0.001[h]
Craving for cocaine (0-70)	-	18.92 (9.01)	18.97 (10.55)	t = 0.02[g]	91	0.98
ADHD-SR sum score (0-22)	7.74 (4.75)	13.39 (9.11)[f]	15.50 (9.16)[f]	F = 13.88[d]	2/156	<0.001[h]
BDI sum score (0-63)	4.42 (4.38)	7.70 (6.76)[f]	10.34 (7.58)[f]	F = 10.73[d]	2/156	<0.001[h]
Hair color (n) (black/brown/blonde/dyed)	4/54/3/2	6/64/3/1	6/21/2/0	χ^2 = 5.94[a]	6	0.43

Sex, smoking,and haircolor in frequency data.
ADHD, attention-deficit/hyperactivity disorder; ADHD-SR, ADHD Self-Rating Scale; BDI, Beck Depression Inventory; FTND, Fagerströom Test of Nicotine Dependence.
[a]χ^2 test (all groups) for frequency data.
[b]Indicates significant post hoc test (Sidak) vs.control group: p<.05.
[c]Indicates significant post hoc test (Sidak) vs. recreational cocaine user group: p<.05.
[d]Analysis of variance (all groups).
[e]FTND measured in smokers only.
[f]Indicates significant post hoc test (Sidak) vs.controlgroup: p<.01.
[g]Independent t test (cocaine users only).
[h]Significant p values.

Table 2. Pattern and amount of drug use: Results of the Psychotropic Drug Interview, urine toxicology, and hair samples

	Control group (n=66)	Recreational cocaine users (RCU, n=64)	Dependent cocaine users (DCU, n=29)	Value[a]	df/dferr	*p*
Cocaine						
times per week[b]	-	1.11 (1.08)	2.85 (2.66)	F = 20.14	1/91	<0.001[h]
grams/week[b]	-	1.18 (1.46)	8.32 (16.15)	F = 12.48	1/91	<0.001[h]
years of use	-	6.08 (3.93)	10.35 (6.91)	F = 14.30	1/91	<0.001[h]
maximum dose (24h)	-	3.27 (2.37)	10.08 (8.49)	F = 35.51	1/91	<0.001[h]
last consumption (days)	-	24.70 (35.99)	28.39 (38.19)	F = 0.19	1/91	0.66
cumulative dose (grams)	-	500.61 (734.45)	7211.70 (10503.26)	F = 26.19	1/91	<0.001[h]
urine toxicology (pos/neg)	-	13/51	13/16	χ^2 = 5.73	1	0.02[h]
hair sample (pg/mg)						
cocaine	-	2780.94 (4695.09)	19967.93 (33082.52)	F = 16.75	1/91	<0.001[h]
benzoylecgonine	-	565.51 (932.77)	4313.62 (7531.84)	F = 15.53	1/91	<0.001[h]
ethylcocaine	-	271.83 (312.28)	1879.31 (3721.96)	F = 11.91	1/91	<0.001[h]
norcocaine	-	63.45 (101.19)	501.64 (739.20)	F = 21.87	1/91	<0.001[h]
MDMA						
tablets/week[b]	-	0.09 (0.27)	0.42 (1.86)	F = 1.91	1/91	0.17
years of use	1.60 (11.31)	2.51 (3.81)	2.97 (5.35)	F = 0.37	2/156	0.69
last consumption (days)[c]	-	66.33 (83.34) (n=20)	77.20 (45.91) (n=8)	F = 0.12	1/26	0.73
cumulative dose (tablets)	0.61 (1.86)	37.70 (92.94)	139.10 (400.50)[d,e]	F = 6.00	2/156	<0.01[h]
hair sample (pg/mg)	2.74 (16.24)	627.24 (1660.04)[d]	240.86 (663.88)	F = 5.15	2/153	<0.01[h]
Cannabis						
grams/week[b]	0.53 (1.47)	0.87 (2.01)	1.85 (4.84)	F = 2.63	2/156	0.08
years of use	5.68 (7.32)	7.56 (5.65)	10.35 (10.66)[f]	F = 4.02	2/156	0.02[h]
last consumption (days)[c]	37.35 (52.23) (n=32)	20.67 (30.84) (n=43)	24.94 (30.72) (n=17)	F = 1.66	2/89	0.20
cumulative dose (grams)	426.73 (903.46)	1082.74 (1780.72)	4491.36 (7478.60)[d,g]	F = 14.84	2/156	<0.001[h]
urine toxicology (pos/neg)	12/54	23/40	11/18	χ^2 = 6.58	2	0.04[h]
Amphetamine						
grams/week[b]	-	0.08 (0.21)[d]	0.01 (0.04)	F = 2.58	1/92	0.11
years of use	0.01 (0.06)	1.64 (2.99)[d]	1.48 (3.23)[f]	F = 8.87	2/156	<0.001[h]
last consumption (days[c]	-	63.10 (52.08) (n=24)	93.06 (74.12) (n=5)	F = 1.19	1/27	0.29
cumulative dose (grams)	0.17 (1.44)	22.58 (59.01)[f]	16.70 (61.01)	F = 4.10	2/156	0.02[h]
hair sample (pg/mg)	0.9 (7.56)	75.47 (259.52)	44.31 (158.97)	F = 2.73	2/153	0.07
GHB						
cumulative dose (pipettes)	-	1.79 (9.77)	1.14 (2.89)	F = 0.12	1/91	0.73
Hallucinogenes						
cumulative dose (times)	1.80 (7.13)	6.78 (15.14)	8.07 (15.37)	F = 3.69	2/156	0.03[h]
Alcohol						
grams/week[b]	118.83 (126.58)	174.96 (118.65)	196.09 (286.64)	F = 2.97	2/156	0.05
years of use	13.83 (9.61)	10.84 (5.08)	14.73 (9.80)	F = 3.22	2/156	0.04[h]
Nicotine						
cigarettes per day (CPD)[b]	9.03 (9.49)	11.96 (8.33)	15.60 (14.20)[f]	F = 4.43	2/156	0.01[h]
years of use	9.52 (9.68)	9.53 (6.34)	15.78 (11.33)[d,g]	F = 5.91	2/156	<0.01[h]

Means and standard deviations in parentheses. Consumption per week, duration of use, and cumulative dose are averaged within the total group.
GHB, gammahydroxybutyrate; MDMA, methylenedioxymethamphetamine.
[a]Analysis of variance or χ^2 test for frequency data.
[b]Average use during the last 6 months.
[c]Last consumption is averaged only for persons who used the drug in the last 6 months. In this case, sample size(n) is shown.
[d]Significant post hoc test (Sidak) vs.control group: p<.01.
[e]Significant post hoc test (Sidak) vs. recreational cocaine users group: p<.05.
[f]Significant post hoc test (Sidak) vs. control group: p<.05.
[g]Significant post hoc test (Sidak) vs.recreational cocaine users group: p<.01.
[h]Significant p values.

3.4.2 PPI

A mixed-design ANCOVA (SOA*group) revealed a significant main effect for SOA ($F(3,459)=9.50$, $p<.001$) and a significant between subjects effect for group ($F(2,153)=4.58$, $p<.01$) (**Figure 1**). The main effect of SOA reflects the nature of PPI to increase with rising SOA (54). The interaction of SOA*group was not significant ($F(6,459)=.97$, $p=.44$). Sidak-corrected pairwise comparisons showed a significantly increased mean %PPI for RCU ($p<.05$, $d=.38$) and DCU ($p<.05$, $d=.67$) compared with controls, whereas RCU and DCU did not differ from each other ($p=.94$, d=.12). A significant effect was found for the covariate sex ($F(1,153)=4.95$, $p<.05$) (women in follicular phase and men>women in luteal phase), whereas the effects of age ($F(1,153)=1.74$, $p=.19$) and smoking status ($F(1,153)=.64$, $p=.43$) did not reach significance.

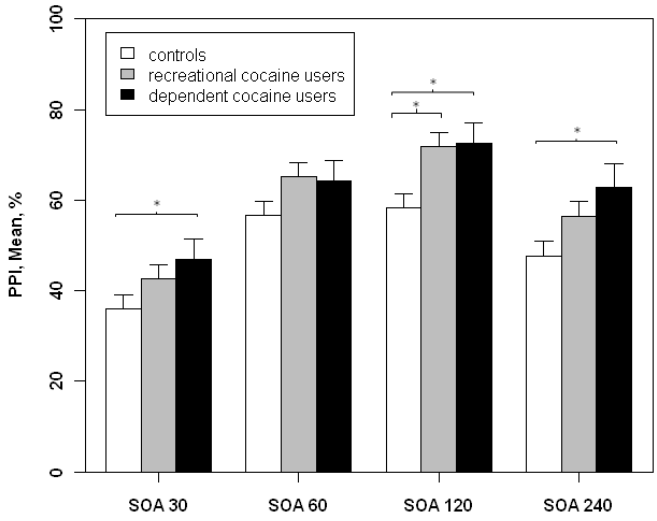

Figure 1. Mean percent prepulse inhibition (PPI) in recreational (n=64) and dependent cocaine users (n=29) and healthy control participants (n=66). Recreational and dependent cocaine users show significantly increased PPI. Error bars refer to SEM. *Significant difference between groups (p<0.05). SOA, stimulus onset asynchrony.

3.4.3 Startle reactivity and habituation

Analyses of covariance performed for groups did not show significant differences in startle reactivity measures (all *p*>.42), although DCU showed slightly decreased startle reactivity compared with controls (*d*=.36, *d*=.43, respectively) (**Table 3**). Furthermore, no significant differences between groups were found for the mean of PPA trials and no-stimulation trials (all *p*>.53). Running the PPI analysis with and without covarying for PA first block yielded similar results.

A mixed-design ANCOVA (PA trials block 1-4*group, smoking status, sex, age, and startle reactivity in block 1 as covariates) did not reveal a significant block*group interaction (F(6,411)=.40, *p*=.85), indicating that habituation did not differ between groups (**Figure S1** in **Supplement 1**). Furthermore, %habituation did not differ between groups across blocks and neither did the linear gradient coefficient *b* (all *p*>.44) (**Table 3**).

Table 3. Startle Reactivity, Mean %PPI, and Habituation

	Control group (n=66)	Recreational cocaine users (RCU, n=64)	Dependent cocaine users (DCU, n=29)	Value[a]	df/dferr	p
PA first block (μV)	121.52 (103.54)	120.72 (68.46)	91.19 (61.15)	F = 0.70	2/153	0.50
PA mean across blocks (μV)	82.00 (61.23)	76.74 (51.13)	58.57 (45.29)	F = 0.88	2/153	0.42
PPA mean (μV)	6.60 (6.72)	6.40 (5.39)	5.28 (3.12)	F = 0.31	2/153	0.74
Baseline (μV)	3.07 (1.39)	2.96 (1.30)	3.04 (1.34)	F = 0.63	2/153	0.53
%PPI mean across conditions	49.68 (23.02)	57.83 (20.88)[b]	64.15 (15.11)[b]	F = 4.58	2/153	0.01[c]
Habituation						
%habituation block 2-3	20.05 (37.85)	23.17 (31.99)	12.23 (52.26)	F = 0.83	2/153	0.44
%habituation block 2-4	25.68 (41.41)	26.91 (47.11)	32.10 (36.62)	F = 0.32	2/153	0.72
coefficient *b*	-21.68 (28.64)	-21.34 (16.37)	-19.06 (14.48)	F = 0.12	2/153	0.89

Means and standard deviations in parentheses
PA, pulse alone trials; PPA, prepulse alone trials; PPI, prepulse inhibition.
[a]Analysis of covariance including all groups (sex, age, and smoking status as covariates).
[b]Indicates significant post hoc test (Sidak) vs. control group: p<0.05.
[c]Significant p value.

3.4.4 Correlations with cocaine use parameters

In cocaine users, %PPI mean across SOA conditions was significantly correlated with cumulated cocaine use (ln-transformed) (*r*=.23, *p*<.03) and duration of cocaine use (*r*=.22, *p*<.03), indicating an increasing PPI with growing amount and time of use (**Figure S2** in **Supplement 1)**. The effects remained after adjusting for age. Correlations between %PPI mean across conditions and current cocaine use, as well as hair samples, did not reach significance (*p*>.28).

3.4.5 Urine toxicology and drug use

To test the influence of recent cocaine use, cocaine users were divided into users with positive (n=26, range: 268–43219ng/ml, mean: 5438ng/ml, SD: 10599ng/ml) and users with negative urine samples (n=67) and compared with control subjects (n=66). A mixed-design ANCOVA (SOA*group) revealed a significant between subjects effect for group ($F(2,153)=4.83$, $p<.01$). Sidak-corrected pairwise comparisons yielded a significantly increased mean %PPI in users with negative urine samples in comparison to controls ($p<.01$, $d=.50$). The SOA*group interaction did not reach significance **(Figure S3** in **Supplement 1)**.

Analogously, the influence of recent cannabis use was investigated by dividing cocaine users into users with positive (n=34, range: 60–726ng/ml, mean: 120ng/ml, SD: 139ng/ml) and users with negative urine samples for cannabis (n=59). They were compared with controls with positive (n=12, range: 66–426ng/ml, mean: 163ng/ml, SD: 131ng/ml) and controls with negative urine samples (n=54). A mixed-design ANCOVA (SOA*group) revealed a significant between subjects effect for group ($F(3,152)=3.33$, $p<.02$). Sidak-corrected pairwise comparisons yielded still significant differences between users with negative urine samples and controls with negative urine samples ($p<.01$, $d=.51$) regarding mean %PPI (**Figure 2**). The interaction of SOA*group was not significant ($F(9,453)=0.62$, $p>.78$).

An ANCOVA (with age and sex as covariates) of mean %PPI comparing controls and cocaine users stratified for smoking status revealed a significant group effect ($F(3,153)=4.73$, $p<.01$) (**Figure 3**). Non-smoking controls differed significantly from smoking cocaine users ($p<.01$, $d=.90$), while the difference to the non-smoking cocaine users was not significant ($p<.30$) despite the considerable effect size ($d=.63$) due to the small group sizes (n=16 vs. n=19).

A detailed analysis of the influence of the use of MDMA and cannabis on PPI is presented in **Supplement 1**.

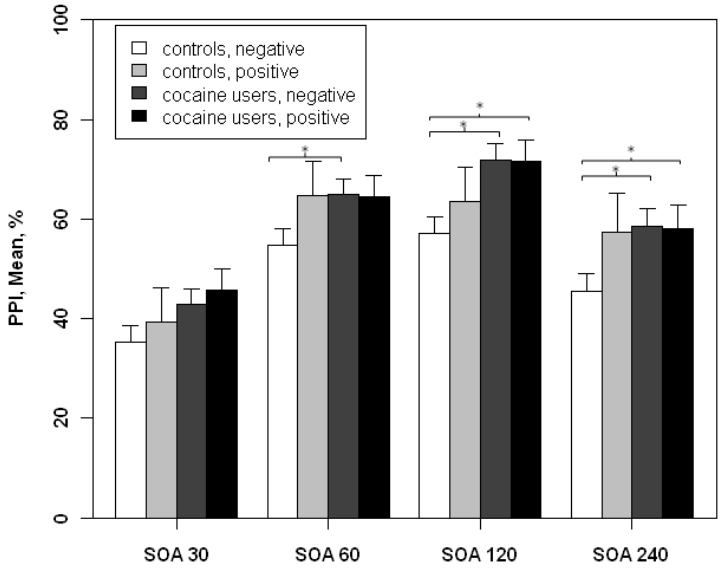

Figure 2. Mean %PPI in control participants with positive (n=12) and negative (n=54) urine samples for cannabis and cocaine users with positive (n=34) and negative (n=59) urine samples for cannabis. Error bars refer to SEM. *Significant difference between groups (Sidak post hoc test: p<0.05). SOA, stimulus onset asynchrony.

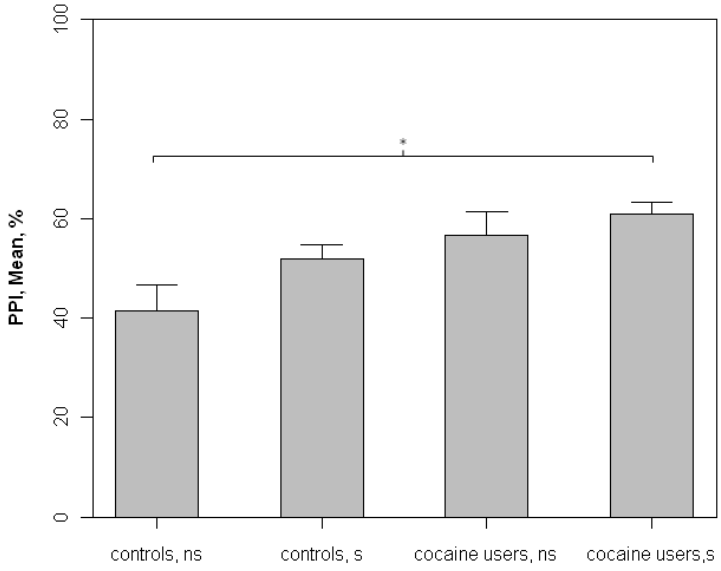

Figure 3. Mean %PPI across SOA conditions in nonsmoking control subjects (controls, ns) (n=16), smoking control subjects (controls, s) (n=50), nonsmoking cocaine users (cocaine users, ns) (n=19), and smoking cocaine users (cocaine users, s) (n=74). Error bars refer to SEM. *Significant difference between groups (Sidak post hoc test: p<0.05). s, smoker; ns, nonsmoker.

3.4.6 Craving and ADHD

In cocaine users, CCQ sum score correlated significantly with %PPI mean across conditions (r=.22, p<.03). Furthermore, when dividing users in a high and low craving group by median split and comparing them with control subjects (**Figure S4** in **Supplement 1**), a mixed-design ANCOVA (SOA*group) revealed a significant main effect for group (F(2,153)=7.05, p<.001). Sidak-corrected pairwise comparisons revealed a significant difference between controls and cocaine users with high craving (p<.001, d=.69). Introducing cumulative cocaine use as a further covariate did not change the results.

The ADHD-SR sum score was significantly correlated with mean %PPI across conditions in cocaine users as well (r=.25, p<.02). To test the influence of ADHD on PPI, cocaine users were divided into cocaine users with and without ADHD according to DSM-IV criteria and compared with controls **(Figure S5** in **Supplement 1)**. A mixed-design ANCOVA (SOA*group) revealed a significant main effect for group (F(2,153)=6.67, p<.002). Sidak-corrected pairwise comparisons revealed that cocaine users with ADHD showed a significantly increased PPI in comparison to controls (p<.001, d=.76). Interestingly, in cocaine users with ADHD PPI enhancement was most pronounced in the SOA 30 and 240 conditions. Cocaine use parameters did not differ between cocaine users with and without ADHD (all p>.47), and ADHD-SR sum score was not correlated with cocaine use parameters. Introducing cumulative cocaine use as a covariate in the mixed-design ANCOVA (SOA*group) revealed similar results (group: F(2,152)=5.57, p<.005).

When comparing cocaine users stratified for an ADHD diagnosis (yes/no) and craving (high/low) with controls (**Figure 4**), a mixed-design ANCOVA (SOA*group) revealed a significant main effect for group (F(4,151)=4.74, p=.001). Controls differed significantly from cocaine users with ADHD and high craving (p<.03, d=.78) and cocaine users without ADHD and high craving (p<.03, d=.66). Cocaine users without ADHD and low craving displayed normal PPI levels. Cocaine use parameters again did not differ between cocaine user groups. Introducing cumulative cocaine use as a covariate did not reveal other results.

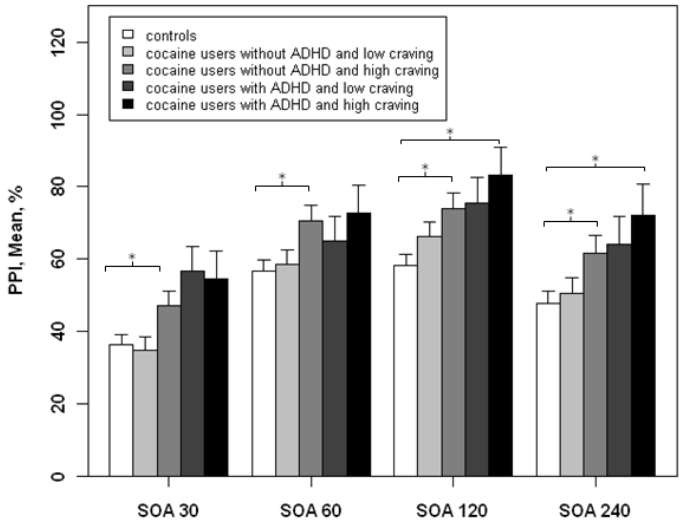

Figure 4. Mean %PPI in cocaine users with ADHD and high craving (n=10), cocaine users with ADHD and low craving (n=12), cocaine users without ADHD and high craving (n=31), cocaine users with ADHD and low craving (n=40), and control subjects (n=66). High craving cocaine users with and without ADHD show significantly augmented PPI, while cocaine users without craving and ADHD display widely normal PPI levels. Error bars refer to SEM. *Significant difference between groups (Sidak post hoc test: $p<0.05$).SOA, stimulus onset asynchrony.

3.5 Discussion

The present study demonstrates that both, RCU and DCU, showed clearly increased PPI levels compared with stimulant-naïve controls. Hair toxicologies and comprehensive psychiatric diagnostics allowed the analysis of a relatively pure and well-described group of cocaine users with little psychiatric comorbidities and without polytoxic drug use. Correlation analyses suggest a positive association between amount and duration of cocaine use and PPI. No significant differences between groups were found with regard to startle reactivity or habituation measures. Furthermore, PPI was significantly augmented in cocaine users with negative urine samples, while users who tested positive for cocaine displayed almost normal PPI levels at least in 60-msec and 240-msec SOA conditions. Finally, cocaine users with ADHD symptoms and high craving showed the strongest increase in PPI, while cocaine users without ADHD but high craving still showed significantly enhanced PPI.

In the sole previous study, Efferen *et al.* (31) reported a statistical trend towards increased PPI in DCU, which was confirmed in the present investigation. Presumably because of the small sample size (10 cocaine users, 9 controls) and the analysis of only three out of six blocks of stimulus trials the effect did not reach significance before (31). In contrast to results obtained in short-term cocaine-withdrawn rats, showing no alterations in PPI (29,30), PPI seems to be sensitive to the lasting effects of cocaine use in humans and the species-specific results might be ascribed to differences in drug dose, route and duration of administration, abstinence period, and pharmacokinetics (55).

The PPI increase most likely reflects alterations in catecholamine neurotransmission:

1) As CSPP circuits regulating PPI and reward circuits, shown to be altered in cocaine users, overlap in the ventral striatum, changes of the striatal DA system are possible (10,11,22,23). This interpretation would be in line with previous studies reporting reduced striatal DA functioning in DCU by applying PET imaging (10-13). DA agonists decrease PPI in rodents (23,56) and humans (55,57). Furthermore, some DA-antagonistic antipsychotics increase PPI in rats (23,56,58,59) and humans (60,61), whereas others like haloperidol have been shown to have no effect or decrease PPI in humans (28,62,63). Thus, the role of striatal DA in the modulation the PPI might be more complex than a simple linear relationship between striatal DA concentration and PPI expression.

2) An increase of thalamic NE transporter density has been reported in DCU (16) and growing evidence suggests an involvement thalamocortical and ventral forebrain NE transmission in the regulation of PPI (25-28,64). Therefore, also dysregulation of NE transmission in CDU as well as RCU might contribute to the current results (17). Changes of the NE system would also be in line with the present and previous findings of slightly reduced startle magnitudes in cocaine users, because pharmacological as well as developmental lesions of the NE system can cause reductions of startle reactivity (65-67).

The finding that PPI was increased exclusively in cocaine users with negative urine samples further support the view that reduced catecholamine levels are responsible for this effect. Users with positive urine samples have used cocaine recently, which is supposed to enhance DA and NE levels and reduce PPI (29,57,68). Furthermore, increased PPI was significantly correlated with amount and duration of cocaine use, which suggests that the alterations in PPI might be substance-induced. PPI seems to be more sensitive to cumulative use compared with recent drug use patterns, as it did not correlate with weekly consumption or hair toxicologies, suggesting that rather long-term changes in catecholamine systems are associated with enhanced PPI. However, although correlations between PPI, cocaine use, and craving may indicate drug withdrawal-induced PPI augmentation, it cannot be ruled out that alterations in PPI and neurotransmitter signaling precede cocaine use and possibly represent a vulnerability to develop cocaine addiction.

Replicating earlier studies, PPI was reduced in women in the luteal phase of the ovarian cycle (51,52). This was explained by an increase in estrogen during the luteal phase, which results in elevated striatal DA release (69). However, as the distribution of women in the luteal and follicular phase did not differ between groups and menstrual cycle combined with sex was included as a covariate, it is unlikely that PPI differences between cocaine users and controls can be attributed to sex or hormonal changes.

In line with previous studies in humans (31) and rodents (30), startle reactivity and habituation were slightly reduced in DCU, but the differences did not reach significance. Though, previous results on startle reactivity have been somewhat inconsistent so far. Whereas Efferen *et al.* (31) reported decreased startle magnitudes in early abstinent cocaine users, Corcoran *et al.* (32) did not report significantly reduced startle reactivity until 40 days of abstinence. In our study, duration of self-reported cocaine abstinence was not correlated with startle reactivity

(*p*=.80). Though, further studies are needed to disclose the influence of abstinence duration on startle reactivity and PPI in cocaine users.

Additionally, we examined the influence of craving and ADHD on PPI, as craving has been associated with dysregulated striatal DA and NE levels (12,17,18,36). Furthermore, dysfunction of NE and DA neurotransmission have been proposed to underlie the pathophysiology in ADHD (34,35,70). ADHD symptomatology and craving both increased PPI in cocaine users compared with controls, even if amount and duration of cocaine use were controlled. Low craving scores were associated with widely normal PPI, independent of ADHD diagnosis. Previous studies reported no significant effect of ADHD on PPI in adults (71,72). However, two studies reported slight but non-significant increases of PPI in untreated (n=13) or treatment-withdrawn (n=22) adults with ADHD (72,73), while stimulant-treated ADHD patients (n=10) displayed somewhat lower PPI levels (72). Perhaps, these studies were underpowered and therefore did not reveal an increase of PPI, even though the study of Feifel *et al.* (71) did not find PPI differences between unmedicated adult ADHD patients (n=20) and controls (n=17). Maybe the PPI-increasing influence of ADHD symptoms shown here only arises from an interaction of cocaine use and underlying ADHD pathophysiology. Taken together, our results might explain why ADHD patients seem to be more vulnerable for addiction than healthy subjects (74) because our results indicate that cocaine using ADHD individuals experience stronger craving symptoms (reflected by maximum PPI increase), possibly due to greater changes catecholamine systems. Additionally, given that we studied only unmedicated cocaine users, cocaine might also be utilized as a self-medication in our users showing ADHD symptoms (74). The need for self-medication might reflect a dysfunctional catecholamine system, which in turn could be more vulnerable for neurochemical plasticity induced by cocaine.

As cocaine users tested in this study showed minimal to moderate co-use of MDMA and cannabis, their influence on PPI was further analyzed. In previous studies, cannabis use did not show significant effects on PPI at least passive attention paradigms just as used here (40,75,76). However, controls with positive cannabis urine toxicology showed a slight even though non-significant increase of PPI, which is in line with several animal studies observing that acute administration of cannabinoid-1 receptor agonists increase PPI (77,78). However, controls and cocaine users with negative cannabis urine samples still significantly differed, indicating that our result cannot be explained by cannabis co-use (**Figure 2**). Moreover, we have previously shown that MDMA users also display elevated PPI levels (40) but here we did

not find an additional increase of PPI in cocaine users with a limited co-use of MDMA. This difference may be explained by either a ceiling effect or by the exclusion of cocaine users with a regular or high use of MDMA in the present study. Given that also MDMA-naïve cocaine users displayed enhanced PPI levels (**Figure S7** in **Supplement 1**) our results cannot be attributed to the MDMA co-use of some users.

The study has some limitations: First, we have no objective measure of the duration of abstinence beyond urine toxicologies but have to rely on self-reports. Thus, we were unable to investigate the true effect of abstinence duration on startle parameters. However, it would have been nearly impossible to control for abstinence in our ambulant and voluntary study setting. Second, although this is one of the first investigations employing hair toxicologies in an electrophysiological study with cocaine users we can only rely on self-reports for illegal drug use prior to 3 to 6 months (depending on hair length), which is nevertheless an inevitable constraint in all studies with illegal drug users (79).

In sum, RCU and DCU showed an increase of PPI that was correlated with duration and amount of cocaine use, as well as the strength of cocaine craving. These data suggest that already recreational use of cocaine is associated with altered catecholamine signaling, which is in line with our previous finding of altered blue-yellow color vision in RCU (19). Moreover, the elevation of PPI was most pronounced in subjects with clinically relevant ADHD symptoms and high craving. PPI might therefore provide a non-invasive, simple, and cheap measure to objectively capture acute stimulant craving symptoms. Finally, our data imply that ADHD might be a critical risk factor for cocaine-induced changes of catecholamine systems.

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3.7 Financial disclosure

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3.9 Supplemental information

Supplemental Methods

Recruitment and Selection Details

Participants were recruited in the Zurich area by means of advertisements in local newspapers, drug prevention and treatment centers, psychiatric hospitals, and internet platforms. Eight-hundred-four potential participants completed an initial telephone screening, whereof 240 subjects participated in the study. Fourty-six participants had to be excluded afterwards because of hair analyses revealing illegal drug use not declared in the interviews (e.g., opioids, excessive MDMA use or polydrug use) or lack of cocaine use. Furthermore, the startle data of 19 participants (11 controls, 8 cocaine users) could not be analyzed because of technical problems/equipment malfunction during the test session. Further 16 participants were excluded due to matching reasons (age, IQ, education, and smoking) between groups (9 controls, 1 cocaine user), startle non-responding (2 controls), and hearing problems (4 cocaine users). Therefore, 159 datasets were included in the analysis. Hair samples were provided by 156 subjects, as hair analysis was not possible by reason of an insufficient amount of hair for three control subjects. Women provided information on the days since their last menstruation. Women in the first 14 days of the cycle were considered in the follicular phase, in the last 14 days in the luteal phase.

Urine and Hair Toxicologies

Urine toxicology analyses comprised the compounds/substances: tetrahydrocannabinol, cocaine, amphetamines, benzodiazepines, opioids, and methadone and were assessed by a semi-quantitative enzyme multiplied immunoassay method using a Dimension RXL Max (Siemens, Erlangen, Germany).

To characterize drug use over the last six months objectively, hair samples were collected and analyzed with Liquid chromatography-tandem mass spectrometry (LC-MS/MS). If participants' hair was long enough, one sample of six cm hair (from the scalp) was taken and subsequently divided into two subsamples of three cm length. The following compounds were assessed: cocaine, benzoylecgonine, ethylcocaine, norcocaine, amphetamine, methamphetamine,

MDMA, MDEA, MDA, morphine, codeine, methadone EDDP (primary methadone metabolite), tramadol, and methylphenidate.

For our routine protocol for drugs of abuse analysis a three step washing procedure with water (2 minutes shaking, 15ml), acetone (2min., 10ml) and finally hexane (2min., 10ml) of hair was performed. Then the hair samples were dried at ambient temperatures, cut into small snippets and extracted in two steps, first with methanol (5ml, 16hours, ultrasonication) and a second step with 3 ml MeOH acidified with 50 µL hydrochloric acid 33 % (3 hours, ultrasonication). The extracts were dried and the residue reconstituted with 50 µL MeOH and 500 µL 0.2 mM ammonium formate (analytical grade) in water. As internal standards deuterated standards of the following compounds were used, added as mixture of the following compounds: cocaine-d3, benzoylecgonine-d3, ethylcocaine-d3, morphine-d3, MAM-d3, codeine-d3, dihydrocodeine-d3, amphetamine-d6, methamphetamine-d9, MDMA-d5. MDEA-d6, MDA-d5, methadone-d9, EDDP-d3, methylphenidate-d9, tramadol-d3, oxycodone-d3, and ephedrine-d3. All deuterated standards were from ReseaChem (Burgdorf, Switzerland), the solvents for washing and extraction were of analysis grade and obtained from Merck (Darmstadt, Germany); LC-solvents were of HPLC grade and were obtained from Sigma Aldrich (Buchs, Switzerland).

The LC-MS/MS apparatus was an ABSciex QTrap 3200 (Analyst software Version 1.5, Turbo V ion source operated in the ESI mode, gas 1, nitrogen (50 psi); gas 2, nitrogen (60 psi); ion spray voltage, 3500V; ion source temperature, 450°C; curtain gas, nitrogen (20 psi) collision gas, medium), with a Shimadzu Prominence LC-system (Shimadzu CBM 20 A controller, two Shimadzu LC 20 AD pumps including a degasser, a Shimadzu SIL 20 AC autosampler and a Shimadzu CTO 20 AC column oven, Shimadzu, Duisburg, Germany). Gradient elution was performed on a separation column (Synergi 4µ POLAR-RP 80A, 150x2.0 with a POLAR-RP 4x2.0 Security Guard Cartridge, (Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of 1mM ammonium formate buffer adjusted to pH 3,5 with formic acid (eluent A) and acetonitrile containing 1mM ammonium formate and 1 mM formic acid (eluent B). The Analysis was performed in MRM mode with two transitions per analyte and one transition for each deuterated internal standard, respectively.

Startle Response Measurement

Electromyographic (EMG) recording was performed in a sound-attenuated room. All participants underwent a short hearing test before the test session to ensure hearing within normal limits. Participants were informed that they would hear broadband noise and bursts over the headphones. They were asked to sit comfortably, stay awake, and keep their eyes open. The eye-blink component of the ASR was measured by an EMG startle system (EMG-SR-Lab; San Diego Instruments, Inc., San Diego, CA). EMG activity was measured from the right *orbicularis oculi* muscle using two silver/silver chloride electrodes. A reference electrode was placed on the glabella. All electrode resistances were less than 10kΩ. The system recorded continuously over the whole session with a sampling rate of 4096Hz. Acoustic startle stimuli were presented binaurally using headphones (TDH-39-P; Maico). The test session started with a 2-min acclimation period of 70dB background broadband noise that was continued throughout the session. Startle stimuli comprised of noise bursts at an 115dB sound pressure level with duration of 40ms, separated by variable intertrial intervals (range: 9-14 seconds, mean: 12 seconds). After an initial pulse-alone trial (PA) the session included a total of 64 trials (56 active and 8 no-stimulation trials) and lasted 13 minutes. Thirty-two pulse trials were preceded by a 20ms prepulse with an intensity of 86dB and a stimulus onset asynchrony (SOA) of 30, 60, 120, and 240ms, resulting in four prepulse (PP) trial conditions. Rise and fall times of all stimuli were less than 1ms. Four startle stimuli were presented at the beginning (block 1) and four startle stimuli at the end (block 4) to assess habituation. Eight no-stimulation (NS) trials and eight prepulse-alone (PPA) trials were recorded to assess baseline EMG activity. PPA trials, NS trials and each of 4 prepulse trial conditions were presented in a pseudorandomized order in blocks 2 and 3.

Supplemental Results

Cannabis and MDMA

Groups of high and low cannabis and MDMA users were created by median split of cumulative use. Cannabis use (cumulative use, duration of use, grams per week) was not correlated with %PPI in the cocaine user group. An ANCOVA comparing the %PPI mean across conditions between cocaine users with no (n=9), low (n=41), and high (n=41) cannabis use did not reveal a significant difference (F (2,85)=.72, *p*=.49) **(Figure S6)**. Introducing cumulative cannabis use as a covariate in the main PPI analysis did not alter the results.

MDMA use measures (cumulative use, duration of use, tablets per week) were also not correlated with %PPI in the cocaine user group (all *p*>.31). The comparison of %PPI mean across conditions between cocaine users with no (n=26), low (n=37), and high (n=30) MDMA use did not reveal a significant difference (F (2,87)=.20, *p*=.82) **(Figure S7)**. Moreover, introducing cumulative MDMA use as a covariate in the main PPI analysis did not change the results.

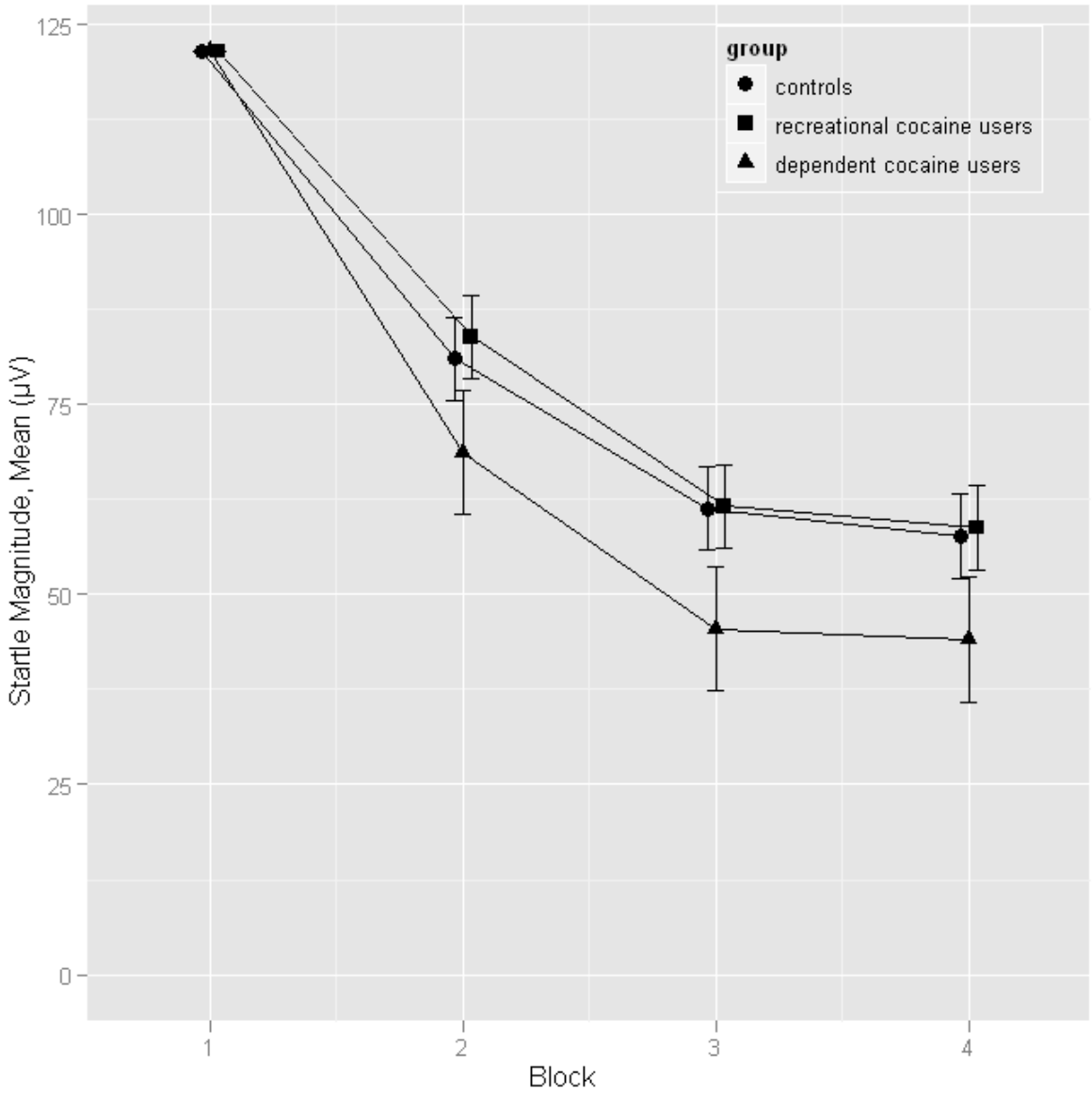

Figure S1: Habituation curve diagrammed as mean amplitude of PA trials in four blocks, corrected for startle reactivity in the first block (means ±SEM) for recreational (n=64) and dependent cocaine uses (n=29), and healthy control participants (n=66). Each block contains four 116-db PA trials.

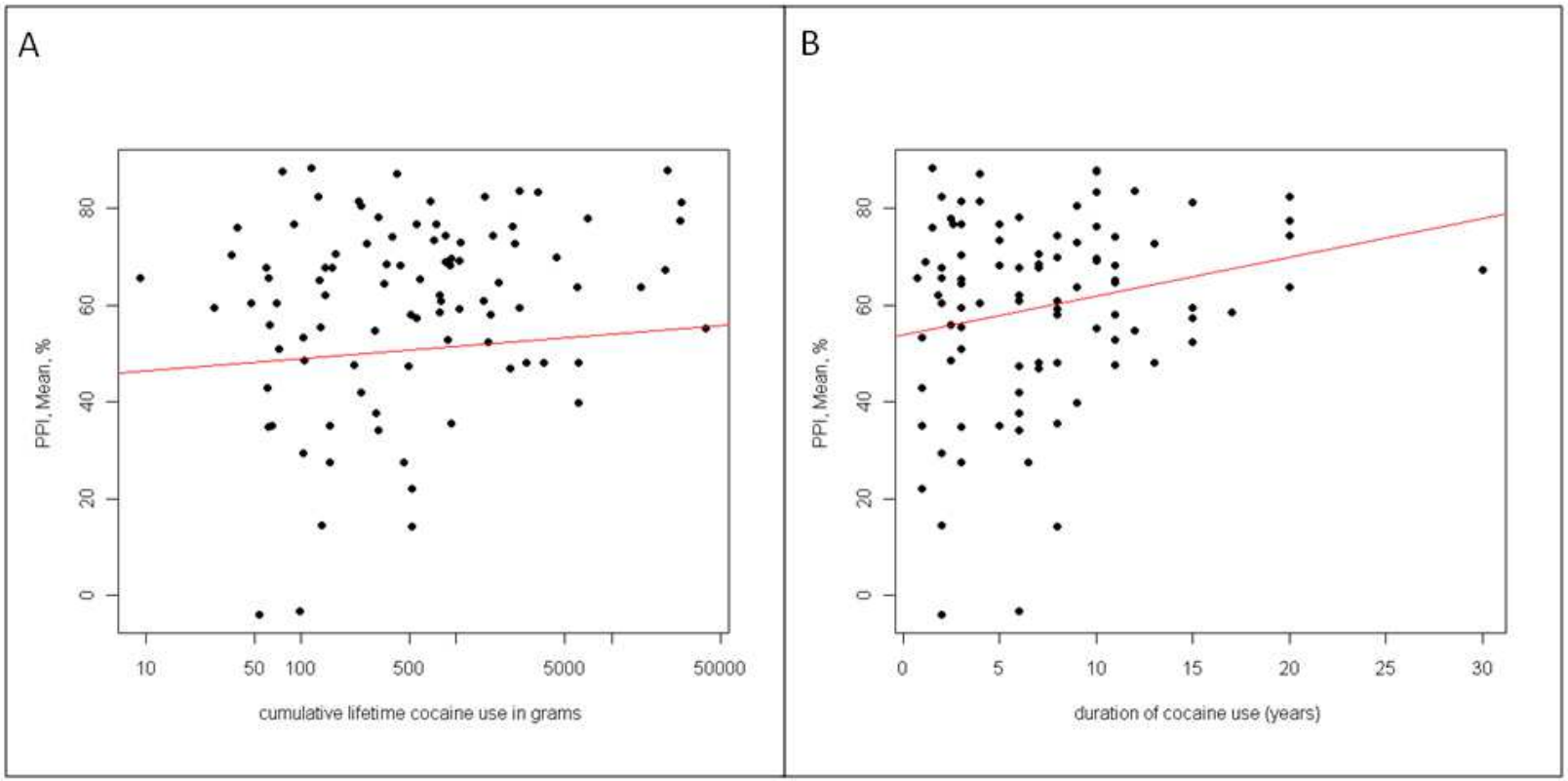

Figure S2: Cumulative lifetime cocaine use in grams (ln-transformed) and mean % prepulse Inhibition (%PPI) across all conditions in cocaine users (r=0.23, p<0.05) (**A**) and duration of cocaine use in years and mean %PPI across all conditions in cocaine users (r=0.22, p<0.05) (**B**). The exclusion of the outlying value reporting 30 years of cocaine use did not change the correlation between duration of cocaine use and %PPI (r=.23, p<.03).

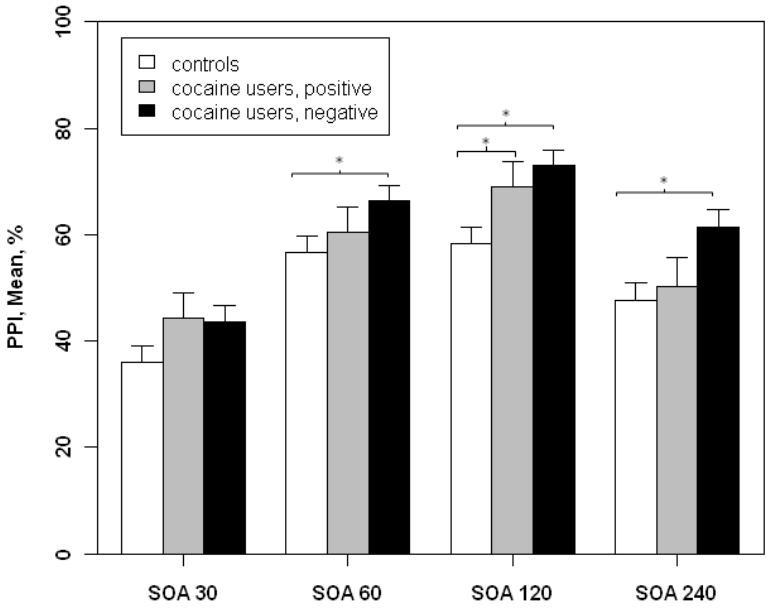

Figure S3: Mean %PPI in cocaine users with positive (n=26) and negative (n=67) urine samples for cocaine, and healthy control participants (n=66). Error bars refer to SEM. *indicates significant difference between groups (Sidak-post hoc test: $p<0.05$). SOA, stimulus onset asynchrony.

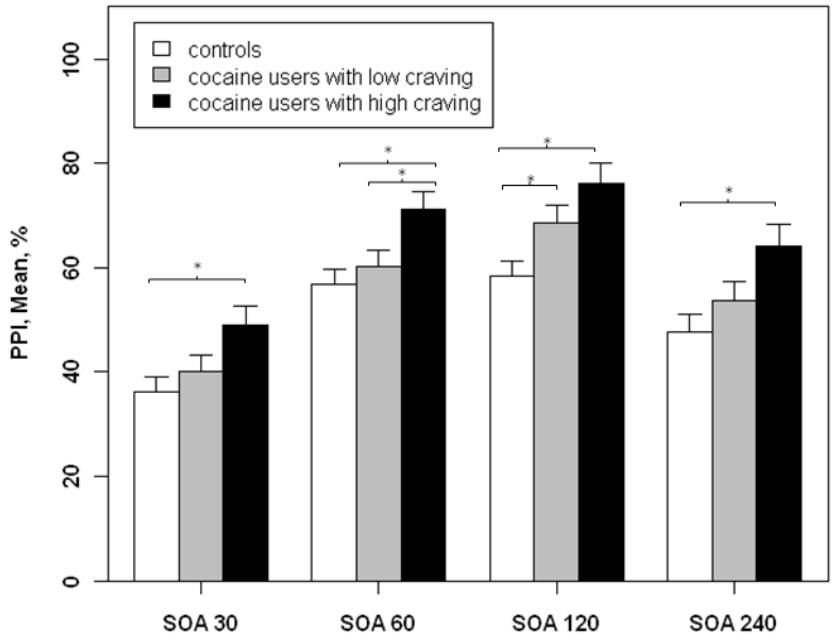

Figure S4: Mean %PPI in low (n=52) and high (n=41) craving cocaine users and controls (n=66). Cocaine users with high craving show increased PPI. Error bars refer to SEM. *indicates significant difference between groups (Sidak-post hoc test: $p<0.05$). SOA, stimulus onset asynchrony.

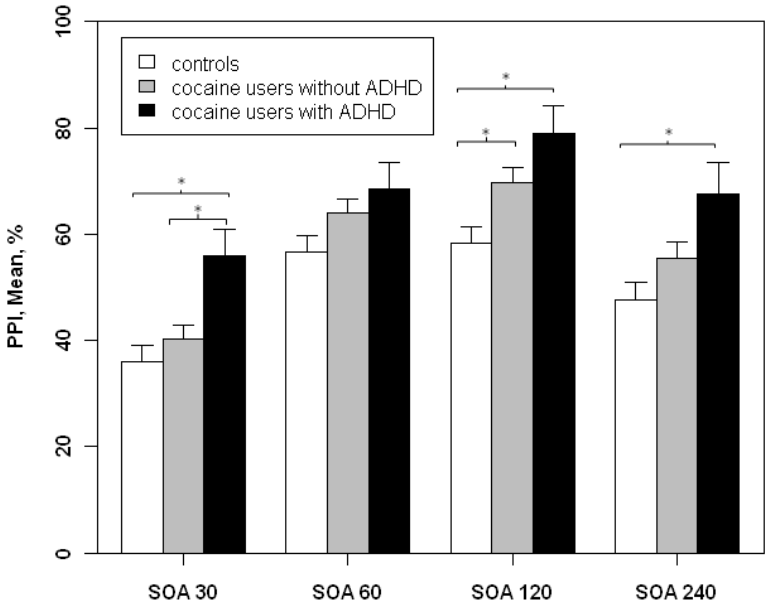

Figure S5: Mean %PPI in cocaine users with Attention-Deficit/Hyperactivity Disorder (ADHD) (n=22) and without ADHD (n=72), and healthy control participants (n=66). Error bars refer to SEM. *indicates significant difference between groups (Sidak-post hoc test: $p<0.05$). SOA, stimulus onset asynchrony.

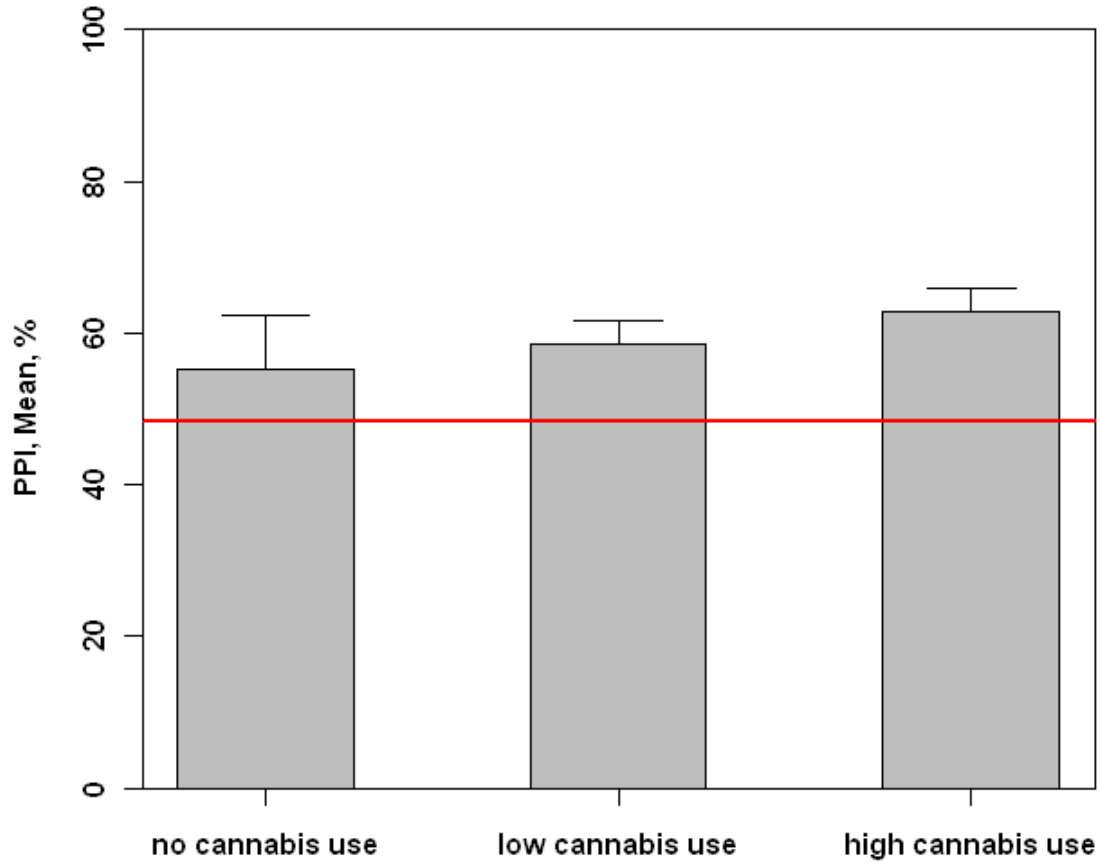

Figure S6: Mean %PPI across conditions in cocaine users with no (n=9), low (n=41) and high (n=41) cumulative cannabis use. The red line represents the PPI level of the controls without cannabis use for comparison. Error bars refer to SEM.

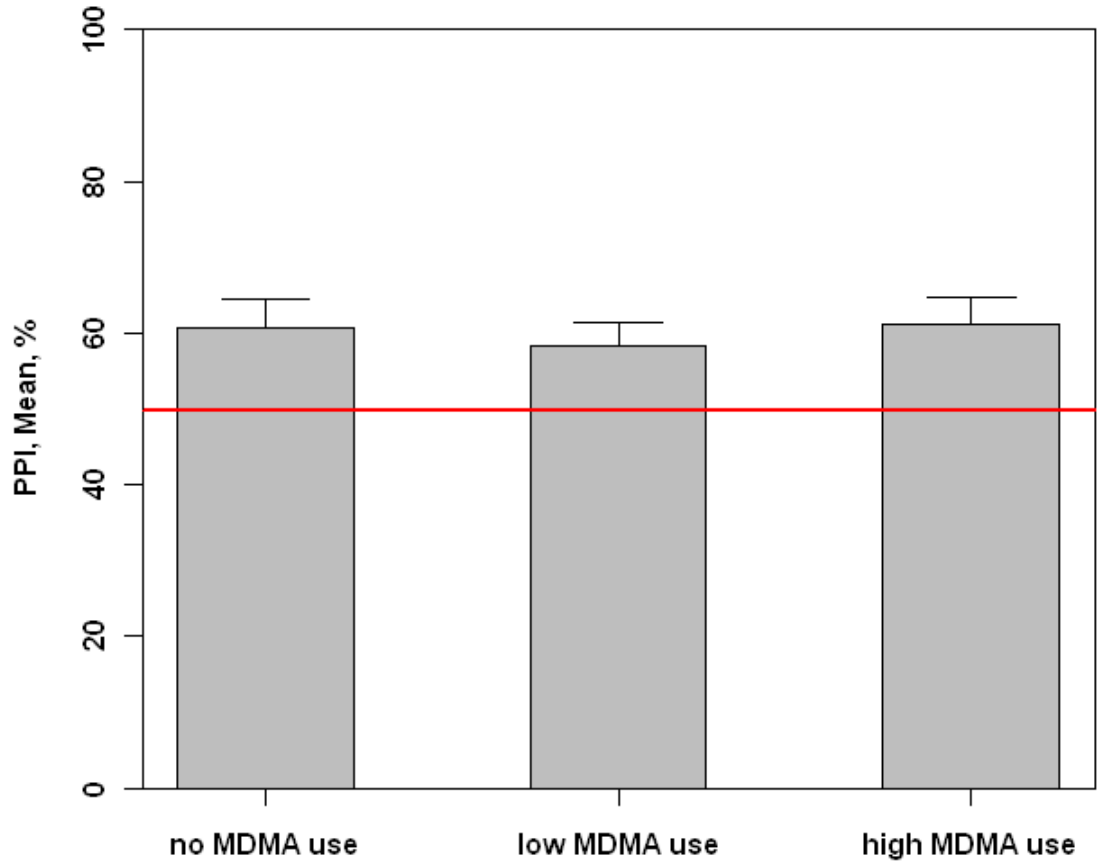

Figure S7: Mean %PPI across conditions in cocaine users with no (n=26), low (n=37) and high (n=30) cumulative MDMA use. The red line represents the PPI level of the controls for comparison. Error bars refer to SEM.

4

Impaired emotional empathy in cocaine users is related to social network deficits

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Personal contribution

KHP gathered and analyzed the data, interpreted the data and wrote the manuscript. BBQ designed the study, helped to interpret the data and revised the first draft of the manuscript. ID designed two of the tests and revised the first draft of the manuscript. LMH, MV, DJ contributed to data acquisition and/or revised the first draft of the manuscript. MRB conducted the hair analyses. ES revised the first draft of the manuscript.

4.1 Abstract

Chronic cocaine users consistently display neurochemical and functional alterations in brain areas involved in social cognition (e.g., medial and orbitofrontal cortex). Although social functioning plays a crucial role in the development and treatment of drug dependence, studies investigating social cognition in cocaine users are lacking. Therefore, we investigated mental perspective-taking ("Theory-of-Mind") and emotional and cognitive empathy in recreational (RCU) and dependent (DCU) cocaine users. Furthermore, we related these measures to real-life indicators of social functioning. One-hundred cocaine users (69 RCU, 31 DCU), and 68 stimulant-naïve healthy controls were tested with the Multifaceted Empathy Test (MET), Movie for the Assessment of Social Cognition (MASC), and Reading the Mind in the Eyes Test (RMET). The Social Network Questionnaire (SNQ) was conducted to assess social network size. Furthermore, participants provided information on committed criminal offences. RCU and DCU showed less emotional empathy compared to controls (MET), whereas cognitive empathy was not impaired (MET, RMET). Additionally, DCU made more errors in mental perspective-taking (MASC). Notably, cocaine users displayed a smaller social network and committed more criminal offences, and both was linked to worse empathy. Moreover, higher cocaine use was correlated with less social contacts and diminished mental perspective-taking, while younger age-of-onset of cocaine use was associated with more pronounced empathy impairment. In conclusion, social cognition impairments in cocaine users were related to real-life social functioning and should therefore be considered in therapy and prevention strategies.

4.2 Introduction

Owing to the high addictive potential and the negative health consequences the use of cocaine is a major public health issue (Degenhardt and Hall, 2012; UNODC, 2011). Cocaine is the second most prevalent illegal drug after cannabis in the United States and Europe (EMCDDA, 2012; HHS, 2011). The current lifetime prevalence for cocaine use in young adults is estimated at 6.3% in Europe (15-34 years) and 13.3% in the United States (18-25 years) (EMCDDA, 2012; SAMHSA, 2011). While most studies on the cognitive and behavioral consequences of cocaine use focus on dependent cocaine users (DCU), a substantial number of cocaine users show a recreational, non-dependent use pattern (EMCDDA, 2012). However, little is known about the neurobehavioral effects of recreational cocaine use, although recent studies suggest neurochemical alterations already in recreational cocaine users (RCU) (Hulka et al., 2012; Preller et al., in press).

Social cognition is an important factor in the development, progress, and treatment of psychiatric disorders, as it was demonstrated for schizophrenia patients (Couture et al., 2006). Analogously, it was proposed that social cognition may play a crucial role in the origin and course of dependence as well as treatment success in stimulant abusers (Homer et al., 2008; Volkow et al., 2011). It has been suggested that addiction impacts brain circuits enabling social functioning by reducing the sensitivity to social reinforcers and enhancing the value of the drug of abuse. Additionally, impaired social cognition may contribute to the decay of social relationships in addicted patients (Volkow et al., 2011), which might have consequences for treatment success, as higher social support is associated with longer abstinence durations (Mutschler et al., 2012).

Surprisingly, studies investigating social cognition in stimulant users are lacking, even though evidence accumulated that chronic cocaine users display neurochemical and functional alterations in brain areas critically involved in several facets of social cognition (Volkow et al., 2011). In particular, marked structural and functional alterations have been reported in the medioprefrontal cortex (MPFC), orbitofrontal cortex (OFC), and anterior cingulate cortex (ACC), temporal cortices including insula, and striatal regions in DCU (Bolla et al., 2004; Ersche et al., 2011; Franklin et al., 2002; Kuhar and Pilotte, 1996; Makris et al., 2008; Volkow et al., 1992). These areas have been associated with social reward (Krach et al., 2010), emotional empathy (Abu-Akel and Shamay-Tsoory, 2011; Fan et al., 2012), cognitive empathy, and theory-of-mind (ToM) (Blair, 2005; Fan et al., 2012; Gallagher and Frith, 2003; Vollm et al.,

2006). Difficulties in understanding, management, and regulation of emotions (Fox et al., 2007; 2011; Kemmis et al., 2007) have indeed been reported in DCU, but social cognitive abilities such as ToM and empathy have not been investigated in cocaine users so far. As effective pharmacological treatments for cocaine addiction are missing (O'Brien, 2005), and social cognition and interaction might be relevant for treatment outcome in addiction, a characterization of social cognition abilities in cocaine users is crucial for the development of more effective prevention and psychotherapeutic treatment strategies.

Therefore, the present study investigates empathy and mentalizing together with real-life social network characteristics in large groups of RCU and DCU, and stimulant-naïve control subjects. Participants completed three tasks to capture different facets of empathy and ToM: the Multifaceted Empathy Test (MET) (Dziobek et al., 2008), the Movie for the Assessment of Social Cognition (MASC) (Dziobek et al., 2006), and the Reading the Mind in the Eyes Test (RMET) (Baron-Cohen et al., 2001). These tasks rely on more ecologically valid stimuli compared to questionnaires or text-based material to meet the demands of everyday social life. The MET is based on the multidimensional model of empathy suggesting two facets of empathy: cognitive and emotional (Blair, 2005; Davis, 1983). The cognitive aspect relates to inferring emotions, the ability to take another persons' perspective, and the understanding of another person's mental state, without necessarily being in an affective state (Baron-Cohen and Wheelwright, 2004; Walter, 2012). Cognitive empathy therefore overlaps with the constructs affective ToM and mentalizing (Frith and Frith, 2003; Walter, 2012). The emotional aspect of empathy describes a person's emotional response to another person's emotional state, i.e., the ability to feel what another person feels (Mehrabian and Epstein, 1972). The MASC was developed as a video-based test of social cognition approximating everyday life demands to further differentiate aspects of cognitive empathy and mentalizing (Dziobek et al., 2006). Finally, the RMET is an established test of attribution and decoding of mental states from the eye region of faces only (Baron-Cohen et al., 2001).

As most previous studies regarding cognitive alterations focused on dependent cocaine use, little is known about the substantial number of cocaine users showing a recreational use pattern. Changes in attention, memory, color vision, and sensorimotor gating have been reported in RCU (Hulka et al., 2012; Preller et al., in press; Soar et al., 2012), but socio-cognitive abilities have not been investigated yet. Notably, examining recreational users holds some further important advantages: RCU are i) not/not yet addicted, ii) less burdened by psychiatric comorbidities (Smith et al., 2009), iii) less likely medicated with psychotropic drugs, and iv) display a reduced amount of polytoxic drug use. This is essential given that polytoxic

drug abuse and psychiatric comorbidities are major confounding factors in addiction research especially with DCU (Degenhardt and Hall, 2012; Prinzleve et al., 2004). Therefore, the application of comprehensive psychiatric diagnostics and the validation of self-reported drug use by hair toxicologies in the present study results in a well-described sample of cocaine users with relatively sparse co-substance use and little psychiatric comorbidities. Consequently, the present study investigates different facets of social cognition in relatively pure recreational and dependent cocaine users compared to an age-, sex-, and IQ-matched group of healthy and psychostimulant-naïve controls. Considering previous imaging findings of altered MPFC, ACC, and striatal function in cocaine users, we expect to find impairments in social cognition in cocaine users presumably linked to real-life social dysfunction such as a smaller social network.

4.3 Methods and materials

4.3.1 Participants

Thirty-one DCU, 69 RCU, and 68 stimulant-naïve control participants were included in the study (for recruitment details see **Supplementary Material Methods 1**). Cocaine dependence was diagnosed following the Diagnostic and Statistical Manual-IV (DSM-IV) criteria (American Psychiatric Association, 1994), with only DCU fulfilling these criteria. Further inclusion criteria for the two user groups were cocaine use of at least 1g per month, cocaine as primary used illegal drug, and a current abstinence duration <6 months. Exclusion criteria for the user groups were use of opioids, polytoxic drug use, and an Axis-I DSM-IV adult psychiatric disorder with exception of cocaine, nicotine, and alcohol abuse/dependence, history of depression (acute major depression was excluded), and ADHD due to their high prevalence in cocaine users. Control subjects were excluded when they displayed any Axis-I DSM-IV psychiatric disorder including ADHD and any form of addiction (except nicotine), and regular illegal drug use (lifetime use <15 occasions) with exception of cannabis. Exclusion criteria for all participants were any neurological disorder or head injury, clinically significant medical diseases, family history of schizophrenia or bipolar disorder, or prescription of drugs affecting the CNS. All participants were asked to abstain from illegal substances for a minimum of three days and from alcohol for at least 24 hours. Self-reports were controlled by urine toxicology and 6-month hair analysis (for details see **Supplementary Material Methods 1 and 2**). The study was approved by the Cantonal Ethics Committee of Zurich (KEK). All participants provided written informed-consent in accordance with the declaration of Helsinki and were compensated for their participation.

4.3.2 Procedure

The present data were collected as part of a larger study on cognitive consequences of cocaine use – the Zurich Cocaine Cognition Study (ZuCo2St) (Preller et al., in press). A Structured Clinical Interview for Axis-I DSM-IV Disorders (SCID-I) was carried out by trained psychologists. Furthermore, participants completed the DSM-IV self-rating questionnaire for Axis-II personality disorders (SCID-II). Participants were asked about number and type of committed criminal offences. Drug use was assessed by means of the Interview for Psychotropic Drug

Consumption (IPDC) (Quednow et al., 2004). The brief version of the Cocaine Craving Questionnaire (CCQ) (Tiffany et al., 1993) was applied to capture current cocaine craving. Smoking habits were assessed with the Fagerström Test of Nicotine Dependence (FTND) (Heatherton et al., 1991). The Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) (Lehrl, 1999), a standardized German vocabulary test, was carried out for estimation of premorbid verbal IQ. The Beck Depression Inventory (BDI) (Beck et al., 1961) was used to assess current depression symptoms, and the ADHD self-rating scale (ADHD-SR) (Rosler et al., 2004) was applied to allow for the diagnosis of ADHD in adulthood according to DSM-IV criteria. Together with a classical neuropsychological test battery and a psychophysiological measurement (as published elsewhere) (Preller et al., in press) the participants completed the tasks described below. Further task details are described in the Supplementary Material Methods 3.

4.3.3 Tests of social cognition

MET: The MET is a reliable PC-assisted test comprising 40 photographs of people in emotionally charged situations (Dziobek et al., 2008). The stimuli depict everyday life situations conveying information on emotional mental states via facial expression, body language, and context. To measure cognitive empathy (CE) subjects are asked to infer the mental state of the person in the photograph and choose which of four words provided along with the picture describes best what the person in the picture is feeling. Explicit emotional empathy (EEE) is assessed by ratings of empathic concern (“How concerned are you for this person?”) on a visual-analogue scale within a range of 1-9 (1=not concerned to 9=very concerned) while viewing the photograph. Implicit emotional empathy (IEE) is measured analogously by arousal ratings (“How calm/aroused does this picture make you feel?”, 1=very calm to 9=very aroused). MET and MASC are implemented in Presentation (Version 14.1, Neurobehavioral Systems, Albany, CA).

MASC: The MASC was developed as ecologically valid and video-based multimodal (visual and auditory input) test of social cognition (Dziobek et al., 2006). Participants are asked to watch a 15-min movie and make inferences about the video characters’ mental states requiring the understanding of emotions, thoughts, and intentions, and concepts such as false belief, faux pas, metaphor, and sarcasm in an everyday-life situation (a dinner with friends). It is paused at 45 times when questions about the actors’ feelings, thoughts, and intentions are asked (“How

is Michael feeling?"). These questions are asked in a multiple-choice format with one correct answer and three distractors reflecting three different types of mistakes: i) insufficient mental state inferences (undermentalizing: reduced ToM), ii) excessive (overmentalizing), and iii) non-mental state inferences (physical causation, no-ToM). Therefore, the MASC provides a sum score for the errors and three subscales for different error types. To control for non-social inference (e.g., about physical events), six control questions are asked during the test.

RMET: The RMET involves inferring the mental state of a person from a photograph which depicts the eye region only (Baron-Cohen et al., 2001). The RMET comprises 36 photographs, for which the participants are asked to choose one out of four mental state descriptors (e.g., jealous, panicked) which describes the person's feelings or thoughts best. The descriptors are varied for each item. A sum score is computed by adding up the number of correctly identified items.

SNQ: The SNQ is based on the social contact circle interview and was designed to evaluate the size of an individual's social network as well as the experienced emotional support and strain by this network (Linden et al., 2007). Participants are required to write down the names of personal contacts in the areas 'household', 'family', 'work or apprenticeship', 'friends', 'neighbors', and 'others'. Only individuals with whom they were in direct contact (via personal encounters, phone calls, emails, or letters) during the previous four weeks should be included. Double entries of contacts are not allowed enabling the calculation of the total social network size. Furthermore, participants are requested to indicate how strongly they feel emotionally supported (e.g., solace and encouragement) by their social contacts on a 5-point scale (1=not at all; 5=very much). Analogously, they specify the emotional strain (e.g., feeling of being rejected) they experience by their social contacts.

4.3.4 Statistical analysis

Frequency data were analyzed by means of Pearson's Chi-square test and quantitative data by analyses of covariance (ANCOVA) or multivariate analyses of covariance (MANCOVA) where appropriate using PASW 18.0 (IBM, Switzerland). In all (M)ANCOVAs, group was introduced as fixed factor. As groups differed in age and years of education (YoE), these variables were introduced as covariates in the analyses. Pearson's product-moment correlations were calculated to relate drug use parameters to social cognition measures. Cumulated cocaine lifetime use was ln-transformed for statistical analyses because of the highly skewed distribution and the resulting deviation from the normal distribution (Shapiro-Wilk $W<.001$ for both user groups). For correlations between illegal drug use and social cognition measures control subjects were excluded to prevent inflating existing correlations. The confirmatory statistical comparisons of all data were carried out on a significance level set at $p<.05$ (two-tailed).

4.4 Results

4.4.1 Demographic characteristics

The groups did not differ with respect to IQ, sex distribution, and smoking status but DCU were older than controls and RCU **(Table 1).** DCU had fewer YoE than controls and RCU. As expected, both user groups scored higher than controls on the BDI and ADHD-SR sum scores as well as on the antisocial personality disorder (PD), and the narcissistic PD scale of the SCID II. Nicotine use (FTND sum score) was higher in DCU than in RCU and controls. Furthermore, RCU and DCU reported more criminal offences than controls.

The hair samples revealed a clear domination of cocaine compared to other illegal drugs as strived for by the inclusion criteria **(Table 2).** DCU showed a more than 8-fold higher concentration of cocaine and metabolites in the hair samples compared to recreational users. A considerable amount of participants were tested positive in urine toxicologies. However, we decided not to exclude them but to investigate the acute and post-acute effects of the drugs.

Table 1. Demographic data (means and standard deviation)

	Control group (n=68)	Recreational cocaine users (n=69)	Dependet cocaine users (n=31)	Value	df/df_{err}	p
Male/female participants (n)	47/21	49/20	24/7	$\chi2 = 0.73$[b]	2	0.70
Age	29.81 (9.13)	28.09 (6.60)	34.81 (10.22)*°°	F = 6.85[a]	2/165	**<0.01**
Years of education	10.62 (1.67)	10.35 (1.91)	9.47 (1.18)**°	F = 4.95[a]	2/165	**<0.01**
Verbal IQ	104.87 (9.66)	103.17 (9.28)	101.81 (11.23)	F = 1.15[a]	2/165	0.32
Smoker/nonsmoker (n)	51/17	55/14	24/7	$\chi2 = 0.43$[b]	2	0.81
FTND sum score (0-10)[d]	2.45(2.26)	3.35 (2.22)	4.79 (2.52)**°	F = 8.58[a]	2/127	**<0.001**
Craving for cocaine (0-70)	-	19.14 (8.64)	20.55 (11.65)	T = 0.67[c]	98	0.50
Antisocial PD (SCID II, 0-15)[e]	2.40 (2.12)	4.39 (3.23)**	4.39 (3.83)**	F = 8.91[a]	2/159	**<0.001**
Narcissistic PD (SCID II, 0-16)[e]	3.06 (2.48)	4.43 (2.56)*	5.11 (3.49)**	F = 7.226[a]	2/159	**<0.001**
ADHD-SR sum score (0-22)	7.56 (5.05)	13.43 (9.65)**	17.00 (8.51)**	F = 17.93[a]	2/165	**<0.001**
BDI sum score (0-63)	3.66 (3.45)	7.83 (6.58)**	11.35 (8.66)**°	F = 19.08[a]	2/165	**<0.001**
Criminal offences[f]						
total	0.54 (0.98)	1.31 (2.07)	1.84 (2.12)**	F = 6.23	2/140	**<0.01**
drug related	0.21 (0.45)	0.65 (1.07)*	1.40 (1.12)**°°	F = 15.43	2/140	**<0.001**
non-drug related	0.33 (0.89)	0.66 (1.74)	0.44 (1.19)	F = 0.68	2/140	0.51
cocaine related	-	0.24 (0.62)*	0.80 (0.71)**°°	F = 19.73	2/140	**<0.001**
non-cocaine related	0.54 (0.98)	1.05 (1.93)	1.04 (1.88)	F = 1.86	2/140	0.16
forfeit	0.47 (0.89)	1.13 (1.98)	1.28 (1.54)	F = 3.76	2/140	**0.03**
conviction	0.07 (0.26)	0.18 (0.61)	0.56 (1.23)**°	F = 5.16	2/140	**<0.01**

Significant p values are shown in bold. Means and standard deviation of means in parenthesis.
FTND, Fagerstrom Test of Nicotine Dependence (in smokers only); PD, Personality Disorder; SCID, Structured Clinical Interview for DSM-IV Disorders; ADHD-SR, ADHD self rating scale; BDI, Beck Depression Inventory
[a]ANOVA (across all groups), [b]$\chi2$ test (across all groups) for frequency data or [c]Independent T-Test (cocaine users only)
[d]FTND measured in smokers only, [e]SCID-II available for 67 controls, 67 RCU, 28 DCU, [f]Criminal offences data available for 57 controls, 62 RCU, 25 DCU
* indicates post-hoc (Sidak) vs control group p<0.05
** indicates post-hoc (Sidak) vs control group p<0.01
° indicates post-hoc (Sidak) vs recreational cocaine users group p<0.05
°° indicates post-hoc (Sidak) vs recreational cocaine users group p<0.01

Table 2. Pattern and amount of drug use: Results of the Psychotropic Drug Interview, urine toxicology, and hair samples

	Control group (n=68)	Recreational cocaine users (n=69)	Dependet cocaine users (n=31)	Value	df/df_{err}	p
Cocaine						
times per week	-	1.10 (1.08)	2.63 (2.46)	T = 4.34[c]	98	**<0.001**
grams/week	-	1.11 (1.43)	6.72 (14.72)	T = 3.15[c]	98	**<0.01**
years of use	-	6.32 (4.06)	9.66 (6.42)	T = 3.16[c]	98	**<0.01**
maximum dose (24h)	-	3.43 (2.45)	9.48 (8.57)	T = 5.42[c]	98	**<0.001**
last consumption (days)	-	27.45 (37.35)	21.62 (35.58)	T = 0.73[c]	98	0.47
cumulative dose (grams)	-	504.32 (751.12)	5325.44 (9505.37)	T = 4.21[c]	98	**<0.001**
urine toxicology (pos/neg)	-	10/58	14/17	$\chi2 = 10.75$[b]	1	**<0.01**
hair sample (pg/mg)						
COC	-	2670.15 (4600.40)	22324.84 (32083.52)	T= 4.97[c]	97	**<0.001**
BEC	-	537.57 (913.16)	4710.65 (7284.00)	T = 4.67[c]	97	**<0.001**
ECO	-	258.71 (298.16)	1918.71 (3583.17)	T = 3.82[c]	97	**<0.001**
NOC	-	61.60 (99.77)	570.16 (733.30)	T = 5.64[c]	97	**<0.001**
MDMA						
pills/week	-	0.08 (0.26)	0.36 (1.79)	T = 1.26[c]	98	0.21
years of use	1.66 (11.15)	2.29 (3.62)	2.92 (5.19)	F = 0.30[a]	2/165	0.74
last consumption (days)	-	111.28 (110.63) (n=22)	81.00 (48.43) (n=8)	T = 0.74[c]	28	0.46
cumulative dose (pills)	0.97 (2.96)	31.91 (86.66)	140.23 (387.41)**°	F = 6.90[a]	2/165	**<0.01**
hair sample (pg/mg)	1.78 (14.46)	509.68 (1462.43)**	218.55 (633.69)	F = 4.58[a]	2/162	**0.01**
Cannabis						
grams/week	0.56 (1.47)	0.95 (2.08)	1.20 (3.68)	F = 1.00[a]	2/165	0.37
years of use	5.45 (6.82)	7.54 (5.91)	9.78 (10.06)*	F = 4.08[a]	2/165	**0.02**
last consumption (days)	33.10 (46.98) (n=30)	19.81 (30.39) (n=45)	66.55 (218.64) (n=17)	F = 1.38[a]	2/88	0.26
cumulative dose (grams)	821.85 (3433.07)	1001.10 (1742.07)	3259.86 (5957.33)**°	F = 5.62[a]	2/165	**<0.01**
urine toxicology (pos/neg)	10/58	15/53	10/21	$\chi2 = 4.04$[b]	2	0.13
Amphetamine						
grams/week	-	0.06 (0.11)	0.04 (0.18)	T = 0.60c	98	0.56
years of use	0.01 (0.06)	1.53 (2.97)**	1.49 (3.12)*	F = 8.85a	2/165	<0.001
last consumption (days)	-	61.25 (51.84) (n=25)	78.38 (75.42) (n=6)	T = 0.67c	29	0.51
cumulative dose (grams)	0.19 (1.42)	17.02 (44.38)*	21.49 (61.88)*	F = 4.59a	2/165	0.01
hair sample (pg/mg)	0.9 (7.39)	84.85 (257.79)*	57.74 (166.85)	F = 3.69a	2/162	0.03
GHB						
cumulative dose (pipettes)	-	1.64 (9.39)	1.24 (2.85)	T = 0.23c	98	0.82
Halluzinogenes						
cumulative dose (times)	1.46 (6.88)	6.45 (14.73)*	6.57 (11.70)	F = 3.83a	2/165	0.02
Alcohol						
grams/week	119.11 (126.73)	176.53 (115.95)	192.32 (256.31)	F = 3.38a	2/165	0.04
years of use	13.23 (9.39)	10.98 (5.22)	13.92 (9.31)	F = 2.01a	2/165	0.14
Nicotine						
cigarettes per day (CPD)	9.18 (9.92)	12.43 (9.00)	15.70 (13.98)*	F = 4.43a	2/165	0.01
years of use	9.05 (9.75)	9.39 (6.38)	13.98 (9.24)*°	F = 4.07a	2/165	0.02

Significant p values are shown in bold. Means and standard deviation of means in parenthesis.
Consumption per week, duration of use, and cumulative dose are averaged within the total group. Last consumption is averaged only for persons who used the drug in the last 6 months.
In this case, sample size (n) is shown.
Pg/mg = picogram/milligram. The hair analysis was performed on two hair samples (each 3 cm in length) per participant capturing drug use over the last six months. Concentrations were averaged over the two samples. If the hair sample was not long enough, only one sample was analyzed (3 cm, 3 months). MDMA = 3,4-methylenedioxy-N-methylamphetamine; methylenedioxy-amphetamine, Cut-off value for cocaine = 500 pg/mg, for amphetamines and MDMA = 200 pg/mg
[a]ANOVA (across all groups), [b]$\chi2$ test (across all groups) for frequency data or [c]Independent T-Test (cocaine users only)
* indicates post-hoc (Sidak) vs control group $p<0.05$
** indicates post-hoc (Sidak) vs control group $p<0.01$
° indicates post-hoc (Sidak) vs recreational cocaine users group $p<0.05$
°° indicates post-hoc (Sidak) vs recreational cocaine users group $p<0.01$

4.4.2 Multifaceted Empathy Test (MET)

A MANCOVA (with age and YoE as covariates) performed for the dependent variables CE, EEE, and IEE revealed a significant main effect for group ($F(6,324)=2.63$, $p<0.02$). Groups differed on the EEE ($F(2,163)=7.94$, $p<0.01$) and IEE scales ($F(2,163)=5.40$, $p<0.01$) **(Figure 1A)**, while no difference was found for CE ($F(2,163)=0.98$, $p=0.38$) **(Figure 1B)**. Sidak-corrected pairwise comparisons revealed a significant difference between controls and RCU ($p<0.02$, $d=0.45$) between controls and DCU ($p<0.05$, $d=0.49$) in EEE. Similarly, in IEE a significant difference was found between controls and DCU ($p<0.01$, $d=0.64$), and controls and RCU ($p<0.05$, $d=0.39$).

As expected, the scores on the antisocial and narcissistic PD scales were significantly correlated with EEE and IEE **(Table 3)**, but introducing antisocial or narcissistic PD as a covariate did not change the main results. Craving did not influence the results as cocaine users with high craving, low craving, and controls did not reveal a significant main effect for group **(Supplementary Material Figure 1)**. Furthermore, controls showed more empathy than both, cocaine users with and without ADHD ($p<0.04$, $d=0.40$-0.68) **(Supplementary Material Figure 2)**, and cocaine users with high and low BDI scores ($p<0.05$, $d=0.39$-0.62) **(Supplementary Material Figure 3)** on the EEE and IEE scale, indicating that even cocaine users with low ADHD and depression symptoms display deficits in emotional empathy.

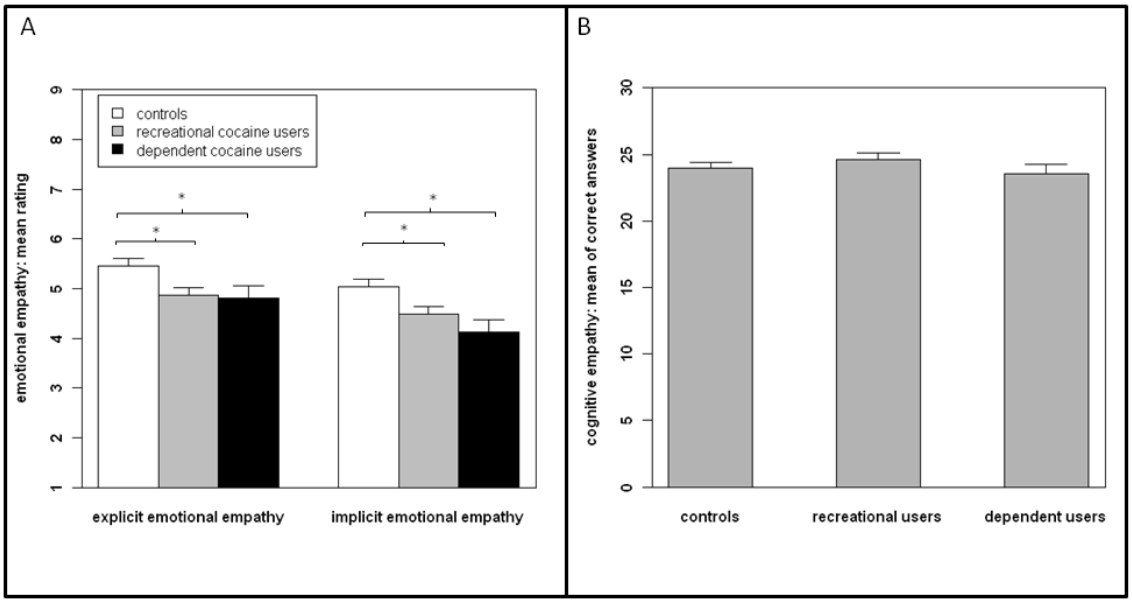

Figure 1. Mean explicit (EEE) and implicit emotional empathy ratings (A) and mean of correct answers on the cognitive empathy (CE) scale (B) of the multifaceted empathy test (MET) for recreational (n=69) and dependent cocaine users (n=31), and healthy control participants (n=68). Recreational and dependent cocaine users show less IEE and EEE than controls. No differences are found for CE. Error bars refer to SEM. *indicates significant difference between groups (p<.05).

Table 3. Correlations between test outcomes, real-life measures of social functioning, and clinical measures in 100 cocaine users, 68 controls and (total n=168).

		MET CE	MET EEE	MET IEE	MASC total errors	RMET Sum score	SNQ total network size	BDI sum score	ADHD-SR sum score	Antisocial PD (SCID II)	Narcissistic PD (SCID II)
MET EEE	controls										
	cocaine user										
	total										
MET IEE	controls		**0.91**								
	cocaine user		**0.85**								
	total		**0.89**								
MASC total errors	controls	-0.29									
	cocaine user	**-0.35**									
	total	**-0.32**									
RMET Sum score	controls	0.24			**-0.40**						
	cocaine user	**0.36**			**-0.35**						
	total	**0.30**			**-0.38**						
SNQ total network size	controls										
	cocaine user										
	total		0.18		-0.16	0.16					
BDI sum score	controls										
	cocaine user				0.23						
	total			-0.16	**0.21**		-0.20				
ADHD-SR sum score	controls					0.24		**0.51**			
	cocaine user				**0.28**			**0.53**			
	total		**-0.20**	-0.15	0.18		-0.17	**0.60**			
Antisocial PD (SCID II)	controls			**-0.32**							
	cocaine user		-0.22			-0.24			0.21		
	total		**-0.28**	**-0.29**		**-0.21**			**0.30**		
Narcissistic PD (SCID II)	controls		**-0.31**	-0.28				0.26	**0.44**		
	cocaine user								**0.38**	**0.40**	
	total	-0.19	**-0.24**				-0.17	**0.28**	**0.45**	**0.40**	
Criminal offences total	controls										
	cocaine user									**0.43**	
	total			**-0.17**					**0.21**	**0.44**	

Correlations with p<0.01 are shown in bold, correlations with p>0.05 are not shown
ADHD-SR, ADHD Self-Rating scale; BDI, Beck Depression Inventory; PD, Personality Disorder; SCID, Structured Clinical Interview for DSM-IV Disorders; SNQ, Social Network Questionnaire, MET, Multifaceted Empathy Test; CE, Cognitive Empathy; EEE, Explicit Emotional Empathy; IEE, Implicit Emotional Empathy; MASC, Movie for the Assessment of Social Cognition; RMET, Reading the Mind in the Eyes Test; CCQ, Cocaine Craving Questionnaire.

The impact of the age of cocaine use onset (AoO) was investigated by a MANCOVA (additionally corrected for cumulated cocaine use), with cocaine users split according to their AoO (AoO≤18: n=27, AoO>18, n=73) compared to controls (n=68). This revealed a main effect for group ($F(6,322)=3.18$, $p<0.01$) with groups differing in EEE ($F(2,162)=10.41$, $p<0.01$) and IEE ($F(2,162)=15.53$, $p<0.001$). Controls differed from cocaine users with early AoO on the EEE and IEE scale (all $p<0.01$, $d=0.90$). On the IEE scale, cocaine users with early AoO differed from cocaine users with AoO≤18 ($p<0.01$, $d=0.63$), whereas cocaine users with AoO>18 did not differ from controls on both scales (all $p>0.21$) **(Figure 2)**. No group differences were found for CE ($F(2,162)=0.36$, $p>0.70$). Moreover, cocaine users with positive urine samples did not differ from users with negative samples ($p>0.69$, $d=0.01$-0.29) on either MET scale.

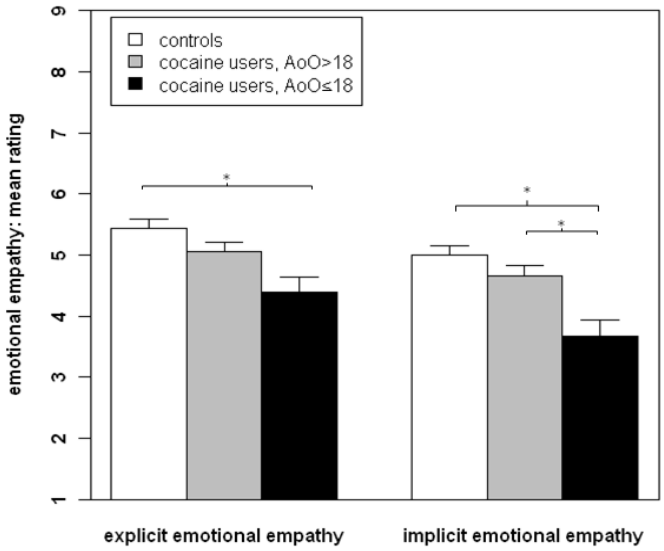

Figure 2. Mean explicit and implicit emotional empathy ratings of the multifaceted empathy test (MET) in cocaine users with an age of onset (AoO) of cocaine use ≤18 (n=27) and >18 (n=73), and controls (n=68). Cocaine users with an AoO≤18 showed less explicit and implicit emotional empathy than controls and less implicit empathy than cocaine user with an AoO >18. Error bars refer to SEM. *indicates significant difference between groups (p<.05)

4.4.3 Reading the Mind in the Eyes Test (RMET)

An ANCOVA (with age and YoE as covariates) did not reveal any significant differences between groups regarding the RMET sum score ($F(2,163)=0.55$, $p>0.58$; mean [SD] for controls: 25.47 [3.82]; RCU: 25.09 [3.43], DCU: 24.16 [3.85]).

4.4.4 Movie for the Assessment of Social Cognition (MASC)

An ANCOVA (corrected for age and YoE) of the MASC total errors yielded a main effect for group ($F(2,164)=3.41$, $p<0.05$). Sidak-corrected pairwise comparisons revealed a significant difference between DCU and controls ($p<0.05$, $d=0.55$), while RCU did not differ from DCU ($p=0.17$, $d=0.46$) and controls ($p=0.97$, $d=0.09$) **(Figure. 3A)**. A MANCOVA with different types of error as dependent variable revealed a significant main effect for group ($F(6,324)=2.41$,

p<0.03), with groups differing on the excessive ToM scale (F(2,163)=4.62, p<0.01). Sidak-corrected pairwise comparisons showed that DCU made more errors with regard to excessive ToM than RCU (p<0.02, d=0.61) and controls (p<0.02, d=0.58). RCU did not differ from controls (p=0.99, d=0.03) **(Figure. 3B).** Groups did not differ with regard to performance in the control items (F(2,163)=1.85, p=0.16) and introducing control items as a covariate in the analysis did not reveal different results. Introducing narcissistic and antisocial PD as a covariate did not change group differences for MASC total errors.

ANCOVAs testing the influence of recent cocaine use, craving, depressive symptoms, and AoO did not reveal significant group differences (all p>0.09), suggesting that these variables did not critically influence the results. However, cocaine users with ADHD performed significantly worse than controls (p<0.01, d=0.72) and cocaine users without ADHD (p<0.02, d=0.63). Cocaine users without ADHD did not differ from controls (p>0.92, d=0.09) (**Supplementary Material Figure 4)**. Nevertheless, introducing ADHD-SR sum score as an additional covariate did not change the main group effect.

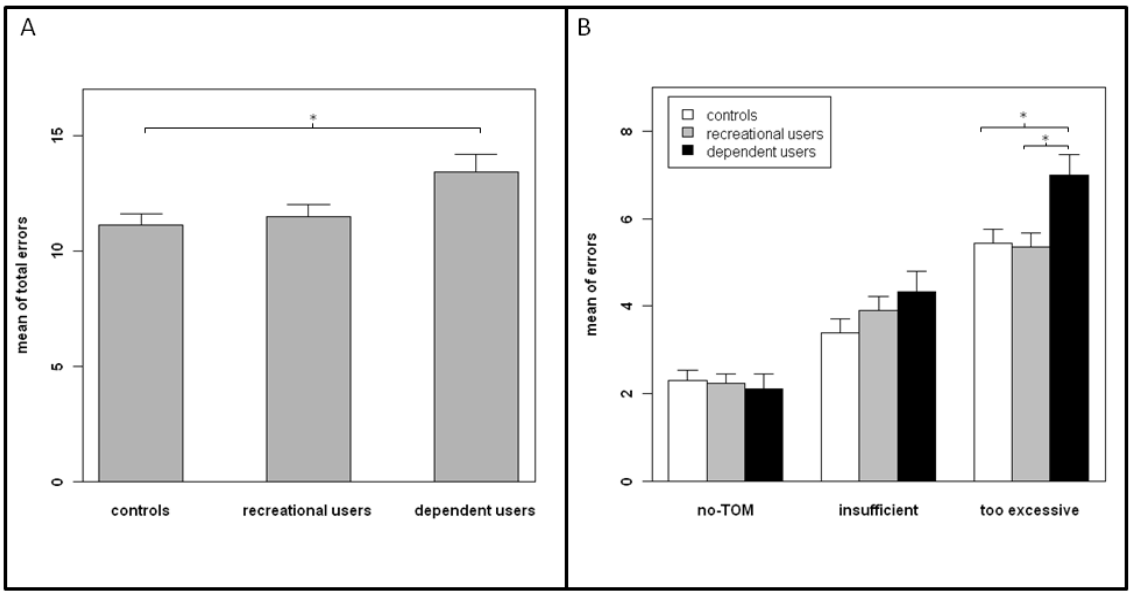

Figure 3. Mean total errors (A) and mean of error types (B) for the assessment of social cognition (MASC) for recreational (n=69) and dependent cocaine users (n=31), and healthy control participants (n=68). Dependent cocaine users made more errors in total than controls and more errors regarding overmentalizing (excessive theory-of-mind [ToM]) than controls and recreational cocaine users. Error bars refer to SEM. *indicates significant difference between groups (p<.05)

4.4.5 Social Network Questionnaire (SNQ)

Social network data were available for 65 controls, 62 RCU and 27 DCU. Differences between groups for the social network size were analyzed with an ANCOVA (corrected for age and YoE), resulting in a significant main effect for group ($F(2,149)=6.34$, $p<0.01$). Both, RCU ($p<0.01$, $d=0.52$) and DCU ($p<0.01$, $d=0.70$) reported a smaller social network than controls **(Figure 4A)**. A MANCOVA with group and scale (emotional support/strain) as fixed factors revealed a significant main effect for scale ($F(1,139)=9.48$, $p<0.01$) and a significant group*scale interaction ($F(2,139)=4.04$, $p<0.02$) **(Figure 4B)**, suggesting that especially DCU experienced less emotional support and more emotional strain than controls.

An ANCOVA (additionally corrected for cumulated cocaine use) with cocaine users split according to their AoO compared to controls (n=65) revealed a significant main effect for group ($F(2,149)=5.39$, $p<0.01$): controls differed significantly from cocaine users with AoO≤18 ($p<0.02$, $d=0.66$) and cocaine users with AoO>18 ($p<0.03$, $d=0.44$) **(Supplementary Material Figure 5)**.

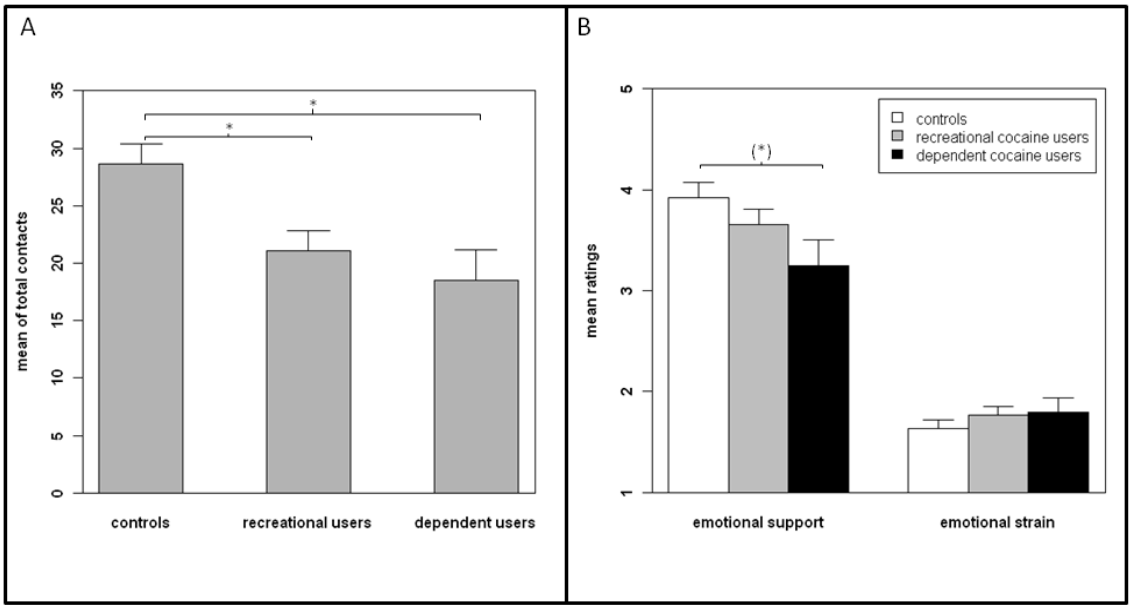

Figure 4. Mean of total contacts (A) and mean of emotional support and strain ratings (B) assessed with the social network questionnaire (SNQ) for recreational (n=62) and dependent cocaine users (n=27) and healthy control participants (n=65). Controls reported more total contacts than recreational and dependent cocaine users. Furthermore, a significant interaction ($p<0.05$) was found for group*emotional support/strain. Error bars refer to SEM. *indicates significant difference between groups ($p<.05$); (*) indicates trend towards significance ($p<.08$)

4.4.6 Correlation analyses

Social network size was significantly correlated with test outcomes on the MET, MASC, and RMET, indicating that impaired social cognition goes along with worse real-life social functioning. Moreover, amount of criminal offences were correlated with the MET IEE score reflecting a negative relationship between emotional empathy and adherence to social norms. As expected, the scores on the antisocial and narcissistic PD scales were significantly correlated with emotional empathy. Interestingly, MET emotional empathy scores and MASC were largely independent from each other, while MET cognitive empathy and MASC were interrelated and both associated with RMET performance **(Table 3)**, indicating that they all share a common cognitive component (e.g., emotion recognition), whereas these more cognitive scores and the emotional empathy measures are largely distinct functions.

Furthermore, the MASC total errors were significantly correlated with cumulative cocaine use (ln-transformed), duration of cocaine use, and cocaine/norcocaine in the hair toxicology, Finally, social network size was negatively correlated with duration and amount of cocaine use **(Table 4)**.

Table 4 Correlations between drug use measures and test outcomes in cocaine users

	MASC total errors	RMET Sum score	SNQ total network size	BDI sum score	ADHD-SR sum score	Criminal offences total
Cocaine						
grams/week						
years of use	0.25		**-0.30**			
cumulative dose (grams)	0.24		-0.25	0.25	0.24	
hair sample (pg/mg)						
COC	0.20					
NOC	0.22					
Cannabis						
grams/week						**0.30**
cumulative dose (grams)						0.23
MDMA						
pills/week		-0.23				
cumulative dose (tablets)		-**0.28**				
Alcohol						
grams/week					0.21	
Nicotine						
years of use	0.22		-0.27			
Cocaine craving (CCQ)				**0.32**	0.23	0.20

Correlations are calculated for cocaine users only, correlations with p<0.01 are shown in bold, correlations with p>0.05 are not shown

Only measures with significant correlations are shown

ADHD-SR, ADHD Self-Rating scale; BDI, Beck Depression Inventory; SCID, Structured Clinical Interview for DSM-IV Disorders; SNQ, Social Network Questionnaire; MASC, Movie for the Assessment of Social Cognition;

RMET, Reading the Mind in the Eyes Test; CCQ, Cocaine Craving Questionnaire.

4.5 Discussion

The present study demonstrates that recreational and dependent use of cocaine is associated with impairments in specific empathy and mentalizing abilities, which interfere with real-life social functioning. Comprehensive psychiatric diagnostics and hair toxicology allowed the analysis of as pure as possible cocaine users with little psychiatric comorbidities. Compared to stimulant-naïve control persons, these cocaine users showed less emotional empathy, whereas cognitive empathy and emotion recognition was not altered. Only dependent users additionally displayed impaired mentalizing functions in the MASC that were correlated with higher cocaine intake. Moreover, both cocaine user groups reported a smaller social network than controls and this real-life indicator of social functioning was significantly correlated with several social cognition measures and amount and duration of cocaine use. In addition, also the number of committed criminal offences was associated with emotional empathy. Taken together, this is, to our knowledge, the first study demonstrating relevant emotional empathy and mentalizing impairment in cocaine users affecting real-life social behavior.

The emotional empathy deficits shown here are in line with previous studies reporting difficulties in the emotion regulation subscale of the Mayer-Salovey-Caruso-Emotional-Intelligence-Test in DCU (Fox et al., 2007; 2011). Importantly, decreased emotional empathy but intact cognitive empathy was previously reported also in patients with narcissistic PD (Ritter et al., 2011), whereas patients with autism displayed the reversed pattern (Dziobek et al., 2008; Gleichgerrcht et al., 2012). However, narcissistic symptoms, often comorbid with cocaine use as also shown here, cannot alone explain the emotional empathy deficits, as statistical control for these symptoms did not change the results. Additionally, narcissistic symptoms were not very pronounced in our sample in comparison to earlier studies, probably due to the strict exclusion criteria regarding psychiatric comorbidities (Ritter et al., 2011). Finally, our finding that cocaine users do not differ from controls in their performance on the RMET is also in line with Kemmis et al.(2007), who reported no differences on the RMET score between controls, occasional, and regular cocaine users as well.

The MASC is a test to sensitively detect subtle deficits in mentalizing abilities in contrast to other less ecologically valid ToM tests (Dziobek et al., 2006). Here, DCU performed worse than controls, while RCU did not show any disturbance. DCU made more errors with regard to

'overmentalizing' (overinterpretive perspective-taking). These results suggest that a subtle deficit in mental perspective-taking may arise from unusual compensation strategies, rather than the loss of cognitive empathy and ToM abilities per se, as they did not make more 'no-ToM' or 'insufficient ToM' errors in the MASC (Sharp et al., 2011). Thus, dependent cocaine users probably try to take the perspective of others but over-interpret social signs. A previous fMRI study showed that the MASC engages the MPFC (Wolf et al., 2010), an area associated with the integration of social information (Van Overwalle, 2009), amongst other regions related to social cognition. As alterations in this brain region were repeatedly reported for cocaine users (Bolla et al., 2004; Ersche et al., 2011; Volkow et al., 1992), it is likely that subtle deficits in mentalizing are associated with MPFC alterations. Therefore, mainly the integration of social information – which is highly necessary to solve the multimodal MASC – might be challenging for dependent users. Furthermore, difficulties in mentalizing were associated with duration of cocaine use, cumulated cocaine use, and cocaine and norcocaine in the hair, suggesting that problems in mental perspective-taking might be partially cocaine-induced. On the other hand, mentalizing deficits may also predispose addictive cocaine use in particular as only dependent individuals showed these deficits.

Cognitive and affective aspects of empathy are behaviorally distinguishable and rely on dissociable, but overlapping, brain networks. In functional imaging studies, emotional empathy engages the ventral striatum, the amygdala, the ACC, the OFC, and the anterior insula (Abu-Akel and Shamay-Tsoory, 2011; Bernhardt and Singer, 2012) ,whereas cognitive empathy depends on the MPFC and the superior temporal gyrus (Vollm et al., 2006). Addicted cocaine users have been reported to show alterations in the ventral striatum, as well as the MPFC, ACC, and OFC (Bolla et al., 2004; Ersche et al., 2011; Kuhar and Pilotte, 1996; Makris et al., 2008; Volkow et al., 1992), and these alterations might be associated with our current finding of impaired empathy. As emotional empathy is already impaired in recreational users, changes in the relevant brain regions (e.g., ventral striatum, OFC, insula), might be more strongly related to cocaine use than changes in areas associated with cognitive ToM (e.g., MPFC). As these regions are also involved in social reward processing (Katsyri et al., 2012) this would support the hypothesis that neuroadaptations in brain reward systems make drug users more responsive to the abused drug and also reduce sensitivity to non-drug reinforcers such as social interaction (Volkow et al., 2011). This might have a negative impact on general social competence and might explain why social consequences such as imprisonment or familial problems may fail to detain drug addicted people from using the drug (Volkow et al., 2011).

Most importantly, our data suggest a relationship between test outcomes and real-life social functioning: Participants showing more empathy and better mentalizing abilities had a larger social network. In addition, social network size was correlated with duration and amount of cocaine use. This implies that cocaine use and the associated deficits in social cognition may have consequences in real life, such as fewer social contacts and less emotional support. Alternatively, a smaller social network might reduce opportunities to adequately learn and practice social abilities, leading to impairments in social cognition and an increase in cocaine use. However, longitudinal studies are needed to disclose causality. Additionally, DCU committed more criminal offences than controls and the number of criminal offences was correlated with social cognition measures. These relationships are especially important, as real-life social behavior and social environment are important factors in onset and therapy of drug use (Ramirez et al., 2012; Shortt et al., 2007). Therefore, therapeutic and prevention approaches focusing on the improvement of social skills and involving the social environment should be intensified to improve the impact of treatment strategies against cocaine addiction.

Furthermore, the age in which cocaine use starts seems to influence emotional empathy and the number of social contacts, with users who began using cocaine before the age 18 showing less empathy than controls. Since we corrected the analyses for cumulated cocaine use, this finding is unlikely to be influenced by longer cocaine use in the early onset group. It is also in line with previous studies, reporting an association between earlier onset of cocaine dependence and elevated motor impulsivity and therefore identifying an early onset of use as particularly dangerous (Prisciandaro et al., 2012). Early onset of drug use may be particularly harmful, because structural, neurochemical, and functional brain development continues during adolescence, which is therefore a period in which the system is highly vulnerable to toxic influences (Crone and Dahl, 2012; Jager and Ramsey, 2008). Moreover, puberty is a critical time period, when complex social-cognitive skills such as mentalizing evolve and social-affective skills such as empathy advance (Crone and Dahl, 2012). Alternatively, social impairments may have preceded drug use and possibly represent a vulnerability to start using drugs. Furthermore, due to their illegal drug use, cocaine users may experience less social support and possibilities to acquire and train social abilities.

One limitation of our study is the lack of an objective measure of the duration of abstinence beyond urine toxicology. Thus, we were unable to investigate the true effect of abstinence

duration on empathy and ToM. However, it would have been nearly impossible to control for abstinence in our ambulant and voluntary study setting.

In sum, our study provides first evidence that dependent and already also recreational cocaine users show deficits in emotional empathy, whereas only dependent users additionally show subtle impairments in cognitive empathy and mentalizing. These results suggest that cocaine users are less emotionally responsive to other people's emotional state and less able to mirror emotions compared to healthy controls. Empathy is crucial to our emotional and social lives (Bernhardt and Singer, 2012) and therefore, these deficits could account for antisocial behavior, especially as social cognition impairments are related to real-life social behavior such as a smaller social network and more criminal offences. The characterization of social cognition abilities in cocaine users is crucial for the development of effective prevention and treatment strategies, as social cognition predicts treatment success in psychiatric disorders (Couture et al., 2006; Homer et al., 2008). Furthermore, it should be considered that treatment approaches requiring strong emotional empathy may not be particularly effective in stimulant users. Thus, treatment success might benefit from trainings designed to improve social cognition and empathy, resembling those designed for schizophrenia patients (Lahera et al., 2012; Rus-Calafell et al., 2012). Finally, prevention strategies should be intensified towards delaying the onset of cocaine use, as a young age of onset is associated with greater deficits in empathy and real-life social behavior.

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4.8 Declaration of interest

All authors declare no conflict of interest.

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4.10 Supplementary material

Methods 1: Recruitment and selection

Subjects were recruited by means of advertisements in local newspapers, drug prevention and treatment centers, psychiatric hospitals, online media, and by word of mouth. Participants were considered eligible for the study if they were aged between 18 and 60 years and had sufficient German language skills. Eight-hundred-four potential participants completed an initial telephone screening, whereof 240 subjects participated in the study. Fourty-six participants had to be excluded afterwards because of hair analyses revealing illegal drug use not declared in the interviews (e.g., opioids, excessive MDMA use) or lack of cocaine use. Further 26 participants were excluded due to matching reasons (age, IQ, education, and smoking) between groups (17 controls, 9 cocaine users). Therefore, 168 datasets were included in the analysis. Hair samples were provided by 165 subjects, as hair analysis was not possible by reason of an insufficient amount of hair for three participants (2 controls, 1 recreational cocaine user). Urine toxicology was not possible for one recreational cocaine user.

Methods 2: Urine and hair toxicologies

Urine toxicology analyses comprised the compounds/substances: tetrahydrocannabinol, cocaine, amphetamines, benzodiazepines, opioids, and methadone and were assessed by a semi-quantitative enzyme multiplied immunoassay method using a Dimension RXL Max (Siemens, Erlangen, Germany).

To characterize drug use over the last six months objectively, hair samples were collected and analyzed with liquid chromatography-tandem mass spectrometry (LC-MS/MS). If participants' hair was long enough, one sample of six cm hair (from the scalp) was taken and subsequently divided into two subsamples of three cm length. The following compounds were assessed: cocaine, benzoylecgonine, ethylcocaine, norcocaine, amphetamine, methamphetamine, MDMA, 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDA), morphine, codeine, methadone EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; primary methadone metabolite), tramadol, and methylphenidate.

For our routine protocol for drugs of abuse analysis, a three step washing procedure with water (2 minutes shaking, 15 ml), acetone (2 min., 10 ml) and finally hexane (2 min., 10 ml) of

hair was performed. Then the hair samples were dried at ambient temperatures, cut into small snippets and extracted in two steps, first with methanol (5 ml, 16 hours, ultrasonication) and a second step with 3 ml MeOH acidified with 50 µL hydrochloric acid 33% (3 hours, ultrasonication). The extracts were dried and the residue reconstituted with 50 µL MeOH and 500 µL 0.2 mM ammonium formate (analytical grade) in water. As internal standards deuterated standards of the following compounds were used, added as mixture of the following compounds: cocaine-d3, benzoylecgonine-d3, ethylcocaine-d3, morphine-d3, MAM-d3, codeine-d3, dihydrocodeine-d3, amphetamine-d6, methamphetamine-d9, MDMA-d5, MDEA-d6, MDA-d5, methadone-d9, EDDP-d3, methylphenidate-d9, tramadol-d3, oxycodone-d3, and ephedrine-d3. All deuterated standards were from ReseaChem (Burgdorf, Switzerland), the solvents for washing and extraction were of analysis grade and obtained from Merck (Darmstadt, Germany); LC-solvents were of high-performance LC grade and were obtained from Sigma Aldrich (Buchs, Switzerland).

The LC-MS/MS apparatus was an ABSciex QTrap 3200 (Analyst software Version 1.5, Turbo V ion source operated in the ESI mode, gas 1, nitrogen (50 psi); gas 2, nitrogen (60 psi); ion spray voltage, 3500V; ion source temperature, 450°C; curtain gas, nitrogen (20 psi) collision gas, medium), with a Shimadzu Prominence LC-system (Shimadzu CBM 20 A controller, two Shimadzu LC 20 AD pumps including a degasser, a Shimadzu SIL 20 AC autosampler and a Shimadzu CTO 20 AC column oven, Shimadzu, Duisburg, Germany). Gradient elution was performed on a separation column (Synergi 4µ POLAR-RP 80A, 150x2.0 with a POLAR-RP 4x2.0 Security Guard Cartridge, (Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of 1 mM ammonium formate buffer adjusted to pH 3.5 with formic acid (eluent A) and acetonitrile containing 1 mM ammonium formate and 1 mM formic acid (eluent B). The analysis was performed in multiple reaction monitoring mode with two transitions per analyte and one transition for each deuterated internal standard, respectively.

Methods 3: Task details

MET: The task is designed to differentiate two facets of empathy: cognitive (CE) and emotional empathy (EE). EE can be sub-divided in explicit emotional empathy (empathic concern, EEE) and implicit emotional empathy (arousal, IEE), where IEE may be less prone to social desirability. CE, IEE, and EEE are assessed for each photograph in a pseudorandomized order. Correct responses in the CE condition are scored as one point, therefore a sum score for CE is

computed, whereas average rating scores are calculated for IEE and EEE. The MET has been shown to detect deficits in CE in patients with autism spectrum disorders (ASD) (Dziobek *et al.* , 2008), and deficits in EE in patients with narcissistic personality disorder (Ritter *et al.* , 2011). Internal consistency of the MET scales ranged from Cronbach's α=0.71 to α=0.92 (Dziobek *et al.* , 2008).

MASC: The MASC has been shown to reliably detect even subtle mindreading difficulties amongst others in healthy individuals of normal IQ (Smeets *et al.* , 2009, Hassenstab *et al.* , 2007), and patients with schizophrenia (Montag *et al.* , 2011), borderline personality disorder (Preissler *et al.* , 2010), and ASD(Dziobek *et al.* , 2006). It has been reported to be more sensitive than other mindreading tests (strange stories task (Happe, 1994), RMET (Baron-Cohen *et al.* , 2001), basic emotion recognition (Ekman and Friesen, 1971)), due to the video-based and realistic nature of the stimuli (Dziobek *et al.* , 2006). The test has a good internal consistency with Cronbach's α=0.84 (Dziobek *et al.* , 2006).

RMET: The photographs can be separated according to the valence of the depicted emotion (neutral: n=16, positive: n=8, negative: n=12) (Baron-Cohen *et al.* , 2001).

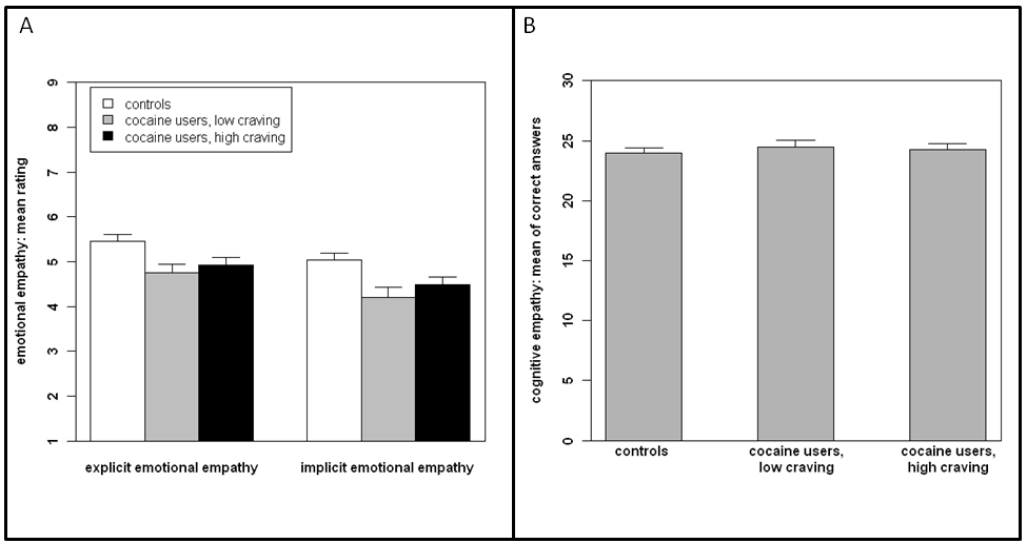

Figure 1. Mean explicit and implicit emotional empathy ratings (A) and mean correct answers on the cognitive empathy scale (B) of the multifaceted empathy test (MET). To test the influence of craving, cocaine users were divided into users with low (CCQ<16, n=41) and high craving (CCQ≥16, n=59) by median split. A MANCOVA (corrected for age and years of education) comparing cocaine users with high craving , low craving, and controls did not reveal a significant main effect for group ($F(6,324)=1.92$, $p<0.08$). Error bars refer to SEM.

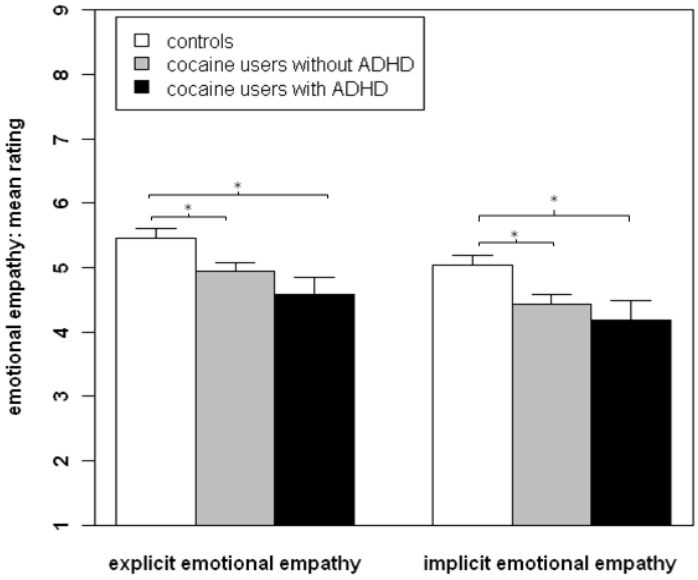

Figure 2. Mean explicit and implicit emotional empathy ratings of the multifaceted empathy test (MET). A MANCOVA (corrected for age and years of education) for ADHD subgroups (cocaine users fulfilling DSM-IV criteria for ADHD (n=22) vs. cocaine users not fulfilling these criteria (n=78) vs. controls (n=68) revealed a significant main effect for group ($F(6,324)=2.84$, $p<0.01$) with groups differing in EEE ($F(2,163)=5.68$, $p<0.01$) and IEE ($F(2,163)=4.91$, $p<0.01$). Controls showed more empathy than both, cocaine users with and without ADHD, on the EEE ($p<0.03$, $d=0.40$-0.68) and IEE scale ($p<0.04$, $d=0.42$-0.59). No significant differences were found between cocaine users with and without ADHD ($p>0.55$, $d=0.16$-0.27). No group differences were found for CE ($F(2,163)=2.53$, $p>0.08$). Cocaine use parameters did not differ between cocaine users with and without ADHD (all $p>.24$). Error bars refer to SEM. * indicates significant difference between groups ($p<.05$).

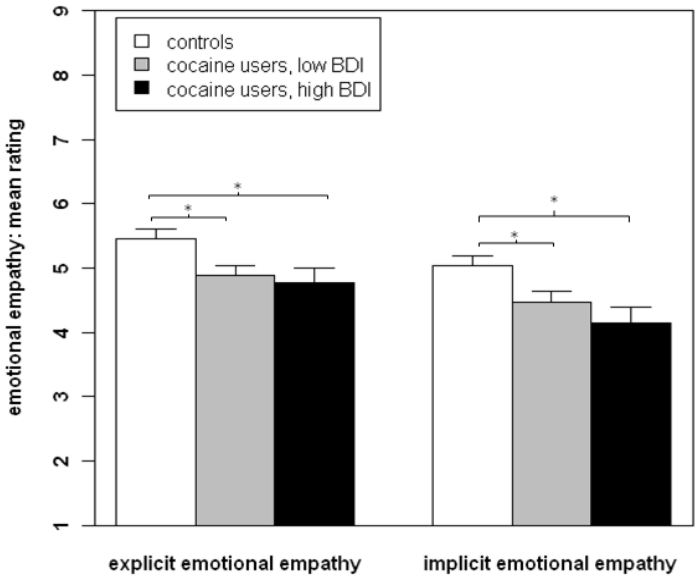

Figure 3. Mean explicit and implicit emotional empathy ratings of the multifaceted empathy test (MET). To test the influence of depressive symptoms, the user groups were split according to a predefined depression criterion (low/high, BDI<11/BDI≥11). A MANCOVA (corrected for age and years of education) for depressive subgroups (low: n=69, high: n=31) comparing them with controls (n=68) revealed a significant main effect for group ($F(6,324)=2.28$, $p<0.04$) with groups differing in EEE ($F(2,163)=5.04$, $p<0.01$) and IEE ($F(2,163)=5.30$, $p<0.01$). Controls differed from both, cocaine users with a high and low BDI score on the EEE ($p<0.03$, $d=0.43$-0.53) and the IEE scale ($p<0.05$, $d=0.39$-0.62). No significant difference was found between cocaine users with low and high BDI scores ($p>0.61$, $d=0.22$-0.43). No group differences were found for CE ($F(2,163)=0.62$, $p>0.54$). Cocaine use parameters did not differ between cocaine users low and high BDI scores (all $p>.05$). Error bars refer to SEM. * indicates significant difference between groups ($p<.05$).

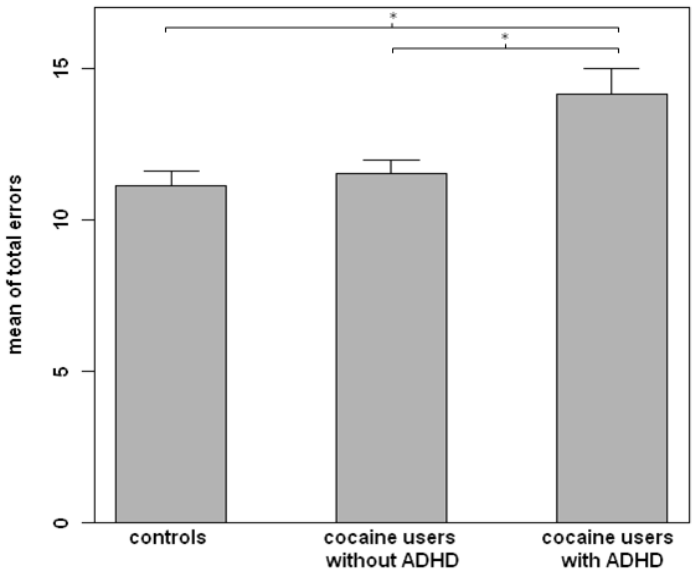

Figure 4. Mean of total errors on the movie for the assessment of social cognition (MASC). To examine the influence of ADHD, cocaine users were divided into cocaine users fulfilling DSM-IV criteria for ADHD (n=22) and cocaine users not fulfilling these criteria (n=78) and compared with controls (n=68). An ANCOVA corrected for age and years of education revealed a significant main effect for group on the MASC total errors ($F(2,163)=4.48$, $p<0.01$) with cocaine users with ADHD performing significantly worse than controls ($p<0.01$, $d=0.72$) and cocaine users without ADHD ($p<0.02$, $d=0.63$). Cocaine users without ADHD did not differ from controls ($p>0.98$, $d=0.09$). Error bars refer to SEM. * indicates significant difference between groups ($p<.05$).

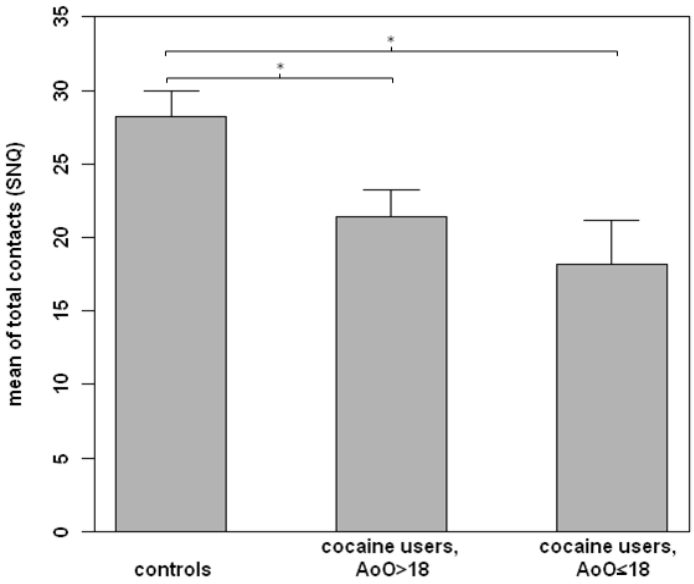

Figure 5. Mean of total contacts assessed with the social network questionnaire (SNQ) in cocaine users with an age of onset (AoO) of cocaine use ≤18 (n=24) and >18 (n=65), and controls (n=65). Cocaine users with an AoO≤18 ($p<0.02$, $d=0.66$) and an AoO>18 ($p<0.03$, $d=0.44$) reported significantly less contacts than controls. Error bars refer to SEM. * indicates significant difference between groups ($p<.05$)

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5

General Discussion

The studies presented in Chapters 2 to 4 were conducted to investigate neural, behavioral and social changes occurring in different stages of drug addiction. The following paragraphs aim to discuss the results of the presented studies in greater detail (Chapters 5.1-5.3), disclose their implications for the addiction models introduced in Chapter 1.1 and for clinical practice such as prevention, treatment and after-care programs (Chapters 5.4 and 5.5), and finally future research questions are discussed (Chapter 5.6).

5.1 Sustained incentive value of heroin cues

Drug dependence, and opiate dependence in particular, has been associated with high relapse rates after treatment (Hser *et al.*, 2001; O'Brien, 1997). According to the drug addiction model proposed by Volkow *et al.* (2011a; 2003; 2011b) and theories of addiction memory (Berke and Hyman, 2000; Hyman and Malenka, 2001; Hyman *et al.*, 2006; Kelley, 2004; Robbins and Everitt, 2002; White, 1996) this vulnerability to relapse might be caused by neuroadaptive effects in memory and motivational networks of the brain. Drug related cues are turned into powerful incentives and may contribute to compulsive drug taking and relapses (Robinson and Berridge, 2000). These theories have been corroborated by studies showing that drug cues are processed as appetitive in nicotine and alcohol dependent persons (Dempsey *et al.*, 2007; Grüsser *et al.*, 2002; Mucha *et al.*, 2000; Rehme *et al.*, 2009). However, it has not been investigated, if heroin-related cues keep their appetitive effects after detoxification and long-term abstinence. This might disclose if changes in memory and reward systems of the brain are lasting even after longer periods of abstinence, and might explain the enduring susceptibility for relapse. Therefore, these issues have been addressed in the experiment presented in Chapter 2 of this thesis.

The analysis of the data presented in Chapter 2 supports incentive theories of drug addiction. We found that heroin-associated cues are processed as appetitive in current heroin users, as heroin-dependent participants in contrast to controls showed a significant reduction in startle amplitude when viewing heroin related cues in comparison with neutral pictures. These results obtained with the implicit measure of affective startle modulation (see Chapter 1.3.1) are supported by explicit ratings of valence. This is in line with previous findings of increased

activations in reward-related brain areas when heroin users are exposed to heroin-related cues (Wang *et al.*, 2011). The incentive effect of heroin-related cues also persists after detoxification therapy in our sample. In alcohol addiction appetitive effects of drug cues have also been measured during detoxification and early abstinence (Grüsser *et al.*, 2002), indicating that alterations in the processing of drug cues outlast therapy at least in alcohol and heroin users. Craving ratings in response to heroin cues also did not change after therapy, proposing a protracted abstinence syndrome (Fatseas *et al.*, 2011; Shi *et al.*, 2007). These strong explicit and implicit appetitive effects of drug cues may increase the vulnerability for relapse after detoxification.

Importantly, the implicit appetitive effects of heroin-related cues can still be found in heroin users after at least one year of abstinence, suggesting that neuroadaptations proposed by addiction models (e.g., Hyman, 2005; Robinson and Berridge, 2000; Volkow *et al.*, 2003; White, 1996) are persistent over longer periods of abstinence in heroin users. This is in line with a previous study , reporting a persistent decrease of DA transporters in the striatum of long-term abstinent heroin users (Shi *et al.*, 2008). Therefore, long-lasting, or maybe even permantent, alterations in the DA system might account for the vulnerability to relapse even after prolonged abstinence (Kalivas and Volkow, 2005; Kelley, 2004).

However, the analysis of explicit ratings of valence and craving showed that former heroin users do not report more craving or pleasure when viewing heroin related cues than controls. As alterations in neural pathways seem to be long-lasting and can still be measured in the implicit reaction to drug cues, this suggests that top-down controlled processes might support the successful abstinence reported by these former heroin users. Therefore, cognitive strategies of devaluating drug cues, which have previously been shown to increase abstinence in smokers (Rose, 2006), may be a promising addition to relapse prevention programs in heroin users. Furthermore, after-treatment programs should be long-lasting and may still be valuable after years of abstinence.

5.2 Increased sensorimotor gating in recreational and dependent cocaine users

In Chapter 2 we showed that neuroadaptations related to drug use can be long-lasting. However, it is not known if already recreational drug use is associated with alterations in neurotransmission. PPI of the acoustic startle reflex has been shown to capture changes in catecholamine neurotransmission (Braff *et al.*, 2001; Zhang *et al.*, 2000): for example antipsychotic medication has been shown to normalize PPI in schizophrenia patients (Csomor *et al.*, 2009; Kumari *et al.*, 2002; Quednow *et al.*, 2006). Reward related areas which have been shown to be altered in dependent cocaine users (Ersche *et al.*, 2011; Volkow *et al.*, 1993; Volkow *et al.*, 1997b) and the CSPP circuits controlling PPI (Koch, 1999; Swerdlow *et al.*, 1999) overlap in the ventral striatum, therefore we assumed that PPI may be a promising and non-invasive measure to capture alterations in neurotransmission associated with cocaine use (see Chapter 1.3.2). However, studies on lasting effects of cocaine use on PPI are scarce. Therefore, in the experiment presented in Chapter 3 of this thesis, it was investigated if changes in neurotransmission in dependent cocaine users can be captured with PPI and whether already recreational cocaine users show alterations in PPI.

The analysis of PPI data revealed that PPI is robustly increased in recreational and dependent cocaine users with amount and duration of cocaine use being positively correlated with PPI increase. Firstly, these results suggest that PPI might indeed be sensitive to alterations in catecholamine systems of cocaine users. Secondly, already recreational cocaine users show modifications of PPI and therefore presumably of catecholamine neurotransmission. This interpretation is supported by our finding that cocaine users with positive cocaine urine samples displayed almost normal PPI levels, given that recent cocaine use increases DA and NE levels (Hutchison and Swift, 1999; Martinez *et al.*, 1999; Ritz *et al.*, 1990). An existing deficit in catecholamine levels may therefore be balanced by the use of cocaine. This is in line with a previous study reporting altered blue-yellow color vision in recreational and dependent cocaine users (Hulka *et al.*, 2012) and suggesting changes of the DA system already at a non-dependent level of cocaine use.

Furthermore, the influence of craving and ADHD symptoms on PPI was examined, since both, ADHD and craving, have been associated with dysregulated DA and NE functioning (Del Campo *et al.*, 2011; Erb, 2010; Howells *et al.*, 2012; Sofuoglu and Sewell, 2009; Volkow *et al.*, 1997b; Volkow *et al.*, 2009). ADHD symptoms and craving increased PPI levels in cocaine users, even when the influence of amount and duration of cocaine use was controlled for. Previous studies did not find alterations or only slight nonsignificant increases in PPI in nontreated adults with ADHD symptoms (Feifel *et al.*, 2009; Hanlon *et al.*, 2009; Holstein *et al.*, 2012). These studies might have been underpowered, or the PPI enhancement in cocaine users with ADHD symptoms might be a result of an interaction of cocaine use and ADHD pathophysiology. Presumably, cocaine users with ADHD symptoms experience more severe craving, which is reflected by the strongest increase in PPI. This may be a result of a predisposed dysfunctional catecholamine system, which could be more vulnerable for cocaine-induced neurochemical plasticity. Therefore, ADHD could be a critical risk factor for stronger cocaine-induced neural alterations and therefore maybe even a faster transition to an addictive state of cocaine use and a greater risk for relapse. Furthermore, while cocaine users with ADHD symptoms and high craving showed the strongest increase in PPI levels, PPI was still significantly enhanced in cocaine users without ADHD but high craving. This suggests that PPI may subserve as a suitable objective measure to capture acute stimulant craving.

5.3 Impaired emotional empathy in cocaine users is related to social network deficits

In the experiment described in Chapter 3 we presented that dependent and already recreational cocaine users probably show alterations in neurotransmission. These neuroadaptations presumably also impact brain areas related to social reward (Abu-Akel and Shamay-Tsoory, 2011; Fan *et al.*, 2012; Gallagher and Frith, 2003; Krach *et al.*, 2010). Accordingly, Volkow *et al.* (2011a) proposed that social behavior might be influenced, as sensitivity to social reinforcers is reduced, while the value of the drug of choice is increased. Furthermore, cocaine-related dysfunctions in brain areas associated with social cognition might have a negative impact on social competence in general. Even though social cognition may have a strong impact the on onset of drug use and treatment success (Homer *et al.*, 2008; Ramirez *et al.*, 2012), studies on social cognition in cocaine users have been lacking. The study presented in Chapter 4 therefore investigates empathy, mentalizing, and real life social behavior in recreational and dependent cocaine users.

As reported in Chapter 4, we could demonstrate for the first time that cocaine users display emotional empathy and mentalizing deficits, which are also reflected in real-life social behavior. Recreational and dependent cocaine users show impairments in emotional empathy, whereas cognitive empathy is not affected. Dependent cocaine users additionally performed worse in a mentalizing task (Movie for the Assessment of Social Cognition, MASC; see Chapter 1.3.3). The MASC has been shown to rely on the MPFC (Wolf *et al.*, 2010). This region has frequently been shown to be altered in cocaine users (Bolla *et al.*, 2004; Ersche *et al.*, 2011; Volkow *et al.*, 1992), therefore deficits in mentalizing in dependent cocaine users may be associated with dysfunctions of the MPFC. As this area engages in the integration of social information (Van Overwalle, 2009), this process might be particularly difficult for dependent cocaine users. These deficits seem to be at least partly drug induced, as worse performance in mentalizing was correlated with higher cocaine intake. However, it might also be possible that social cognition deficits represent a predisposition for more intense cocaine use.

Since only emotional empathy is affected in cocaine users and cognitive empathy is spared, and already recreational users show these deficits, alterations in brain areas related to

emotional empathy (e.g. ventral striatum, OFC; insula (Abu-Akel and Shamay-Tsoory, 2011; Bernhardt and Singer, 2012; Walter, 2012)) might be more strongly associated with cocaine abuse than changes in areas engaged in cognitive empathy (e.g. MPFC (Vollm *et al.*, 2006)). Furthermore, these data suggest that striatal and orbitofrontal areas are already impaired at an early stage of drug use, while dysfunctions of the MPFC occur only later, at a dependent level of cocaine use. Striatal and frontal areas are also related to social reward processing (Katsyri *et al.*, 2012). This corroborates the model by Volkow *et al.* (2011a), which suggests that alterations in the reward system make drug users more responsive to the abused drug, but less sensitive to social rewards.

Empathy is fundamental to our emotional lives and social interactions (Walter, 2012). Our data suggest that deficits in empathy measured with the tests of social cognition used in Chapter 4 are related to real-life social behavior and functioning. Cocaine users with deficits in empathy and mentalizing had a smaller social network and committed more criminal offences. As social network size was associated with duration and amount of cocaine use, this corroborates the hypothesis that cocaine use leads to neuroadaptations and deficits in social cognition, which has consequences in real-life such as a smaller social network. However, a smaller social network might also limit the possibility to learn social abilities leading to the measured deficits and increase the vulnerability to drug use. This is plausible, since the acute effects of cocaine use include enhanced self-esteem and self-confidence in social situations (Dackis and Gold, 1985) and might therefore make cocaine interesting for people with problems in social interaction.

Moreover, we showed that persons who start using cocaine before the age of 18 displayed more deficits in empathy. This is in line with previous studies showing that an early onset of drug use is particularly harmful and for example leads to greater IQ declines and elevated impulsivity (Meier *et al.*, 2012; Prisciandaro *et al.*, 2012). This may be explained by the ongoing brain maturation during adolescence and the continuative development of social skills during this period (Crone and Dahl, 2012; Jager and Ramsey, 2008). Therefore, prevention strategies should emphasize the harmful nature of adolescent drug use for cognition and real-life social functioning.

5.4 Implications for models of addiction and the understanding of substance use disorders

In sum, these results corroborate different aspects of the addiction model proposed by Volkow *et al.* (2011a; 2003; 2011b) and models of addiction memory (Berke and Hyman, 2000; Hyman and Malenka, 2001; Hyman *et al.*, 2006; Kelley, 2004; Robbins and Everitt, 2002; White, 1996) (see Chapter 1.1). The results of the experiments reported in Chapter 2 confirm the close relationship between reward-related learning, memory, and addiction, which has been proposed by theories of drug addiction and addiction memory. Presumably by repeated association of drug cues with the rewarding effect of the drug itself, neuroadaptations result in a hypersensitive DA system in response to drug-associated stimuli (Kelley, 2004; Robinson and Berridge, 1993). Volkow *et al.* (2011a; 2003; 2011b) also proposed that memory circuits are overactive in addicted drug users and assign a higher value to the experience of the drug effects than in non-addicted persons. This contributes to a vicious circle of compulsive drug taking. Our results confirm the role of drug cues as appetitive stimuli that induce craving and may contribute to the continuing use of the drug. Memory systems seem to process drug cues differently in users of the substance than in controls naïve to the drug. This corroborates that drug use exerts an influence on brain systems related to consolidation of experiences. Furthermore, we could broaden these models by showing that the altered processing of drug cues is sustained after detoxification and even after long-term abstinence. This suggests that neuroadaptations in memory systems induced by drug use are long-lasting and may contribute to the vulnerability to relapse even after years of abstinence (O'Brien, 1997). Additionally, we found that despite the implicit reaction to drug cues, the explicit cognitive evaluation of the valence of drug cues returns to normal levels after long-term abstinence. This might indicate, that the control circuit, which is suggested to be weakened in addiction (Volkow *et al.*, 2011a; 2003; 2011b), recovers earlier in abstinence and allows to inhibit drug-cue driven approach behavior and therefore to maintain abstinence.

Furthermore, addiction models described in Chapter 1.1 only cover alterations in a drug dependent state, even though it is important for prevention and therapy to know if already recreational drug use holds the risk of neurochemical alterations. In Chapter 3 we could show that recreational cocaine use is likely associated with altered catecholamine functioning.

Moreover, we demonstrated that further factors such as ADHD symptoms and craving in may contribute to neurochemical plasticity induced by drugs. As described in 5.2 cocaine users with ADHD and craving symptoms showed the greatest increase in PPI, suggesting that cocaine users with ADHD symptoms might also experience more craving. A dysfunctional catecholamine system has been shown to be associated with ADHD symptoms (Del Campo *et al.*, 2011; Howells *et al.*, 2012), thus cocaine users with ADHD might be at risk for stronger cocaine-induced alterations of the brain. This might result in a faster transition to an addictive state and a higher risk for relapse. Therefore, further influencing factors, such as ADHD and potential psychopathology, should be considered in models of addiction. Moreover, we demonstrated that already recreational cocaine users show electrophysiological and behavioral alterations. Therefore, addiction models should also embed recreational drug use, as dysfunctions in catecholamine neurotransmission have been associated with craving and loss of control over drug intake (Martinez *et al.*, 2007; Volkow *et al.*, 1997a; 1997b), and impaired social cognition may increase the vulnerability for drug taking and decrease treatment success (Couture *et al.*, 2006; Homer *et al.*, 2008; Volkow *et al.*, 2011a). Addiction may be a dimensional construct, with neuronal and behavioral consequences already at a non-dependent state of drug use.

The data presented in Chapter 4 support the assumption that substance use is associated with deficits in social behavior (Volkow *et al.*, 2011a). Dependent and recreational drug users show impairments in emotional empathy, which are related to a real-life social functioning such as a smaller network size. These data corroborate models proposing that drug use influences brain areas which are engaged in social cognition (e.g., Hyman *et al.*, 2006; Kalivas and Volkow, 2005; Volkow *et al.*, 2011a). Furthermore, they support the hypothesis that neuroadaptations in brain reward systems increase the perceived value of the abused drug but decrease responsivity to non-drug reinforcers such as social interaction, which also might render drug users insensitive to social consequences such as imprisonment or decline of relationships (Volkow *et al.*, 2011a; Volkow *et al.*, 2009). However, in order to be generalized, social cognition deficits have to be replicated in users of other drugs.

5.5 Implications for clinical practice

The results presented in Chapters 2-4 hold several implications for treatment and prevention of substance use disorders. First of all, in Chapter 2 we found that even after long-term abstinence heroin users still show enhanced implicit appetitive processing of drug cues, which increases the vulnerability for relapse in particular in response to drug-related stimuli. Therefore, after detoxificantion and even after longer periods of abstinence the contact with dug related cues and environments should be very careful. Drug paraphernalia as well as places and people associated with drug use should be either avoided or cognitively devaluated if possible. Trainings of cognitive strategies to reduce the motivational impact of drug cues should be applied during and after detoxification therapy. These strategies have already been shown to successfully enhance abstinence in smokers (Rose, 2006). After detoxification, former heroin users should be motivated to take part in long-lasting relapse prevention programs (Min *et al.*, 2011), as explicit and implicit valence and craving ratings in response to drug cues did not change with detoxification. Long-term support after treatment should be ensured, as sensitization of neural pathways seems to be persistent even after prolonged abstinence. Furthermore, education about this sustained vulnerability to relapse in response to drug cues ought to be part of relapse prevention programs.

Secondly, ADHD symptoms have been identified as a risk factor for increased craving and maybe for enhanced vulnerability to cocaine-induced plasticity. Consequently, cocaine users with ADHD symptoms may be at greater risk to undergo the transition from recreational to addicted use and for relapse. This should be implemented and reported in prevention programs. Furthermore, ADHD symptoms should be considered in therapy. It has to be evaluated if cocaine addicted patients with ADHD might need different pharmacological and therapeutic interventions than cocaine users without ADHD symptoms.

Thirdly, our data suggest that already recreational cocaine users are at risk of neurochemical alterations. These changes are associated with craving and loss of control over drug use (Martinez *et al.*, 2007; Volkow *et al.*, 1997a; 1997b), and deficits in social cognition (Chapter 4), and therefore possibly the devaluation of social stimuli and the loss of social contacts and relationships (Volkow *et al.*, 2011a). Thus, recreational drug use has to be taken seriously and education about these risks should be offered in prevention programs. Interventions and

education in settings of recreational drug use ought to be intensified and may be implemented already at the level of school education.

Fourthly, the deficits in empathy and mentalizing should be considered in therapy and prevention approaches as well. Available pharmacological treatments for cocaine addiction are currently not sufficiently effective (O'Brien, 2005) and treatment concepts such as cognitive behavioral therapy, community reinforcement approach, and contingency management rely on the emotional responsiveness and ability to process social reinforcement of the patients (Moos, 2007). It should be considered that treatment approaches relying on emotional empathy may not be particularly effective in stimulant users. Before these interventions are applied, it may be useful to focus on trainings designed to improve social skills, which might resemble those designed for schizophrenia patients (Lahera *et al.*, 2012; Rus-Calafell *et al.*, 2012). Furthermore, for therapy and prevention strategies it should be considered that threats such as imprisonment, or family and relationship problems might not have the same deterrent effect already in recreational cocaine users.

Lastly, we could show that an early onset of cocaine use might be particularly harmful, as a young age of onset was associated with greater deficits in empathy. Therefore, prevention strategies should be implemented at a reasonably early age and should aim at delaying the onset of cocaine use as long as possible.

5.6 Perspective

With the studies presented in the experimental part of the thesis, we were able to fill gaps in the current knowledge related to the stability of addiction memory, alterations in neurotransmission in recreational cocaine users, and social cognition deficits in cocaine users. However, some open questions remain, which should be addressed in future studies.

First of all, the studies presented in Chapter 2 were mainly conducted with male heroin users. It has still to be evaluated if the results are generalizable to women. Moreover, it should be investigated if trainings to devalue heroin cues are successful in reducing the implicit value and in maintaining abstinence. The efficacy of these trainings ought to be evaluated during detoxification and during after-care relapse prevention programs. Furthermore, we showed that cue reactivity is enhanced in former heroin user who remained abstinent for at least one year. However, it is still not known if alterations in memory systems outlast this time period or are maybe even permanent, as suggested by some models of addiction (e.g., Kalivas and Volkow, 2005; Kelley, 2004). Therefore, former heroin users who were able to maintain abstinence for longer time durations have to be investigated. Moreover, since Savvas *et al.* (2012) reported that methadone treatment influences emotional reactivity, it still has to be investigated if patients in an opioid-maintainance program show different processing of drug-related cues than the abstinent heroin users. Finally, our data suggest that the cognitive evaluation of the valence of drug cues returns to normal levels after long-term abstinence. This might be associated with normal functioning of control circuits. In accordance with addiction models (Volkow *et al.*, 2011a; 2003; 2011b), brain areas such as the PFC seem to be able to inhibit drug-cue driven approach behavior resulting from still dysfunctional memory circuits. This hypothesis should be investigated using imaging methods, to evaluate if certain brain areas recover earlier in abstinence and are therefore supportive in maintaining abstinence.

The studies presented in Chapter 3 and 4, which were conducted with cocaine users, are cross-sectional studies, therefore we cannot draw any conclusions so far about whether the deficits are drug-induced or represent a predisposition and maybe even an increased vulnerability for drug use. Therefore, longitudinal analyses, as strived for by our group, are necessary to disclose causality, and examine if differences in PPI and therefore neurotransmission may have preceded cocaine use. Furthermore, longitudinal studies will offer the possibility to examine if

social cognition deficits are cocaine induced and have consequences in real-live social behavior or whether the smaller social network and lack of support prevented a normal development of social cognition abilities and therefore promoted drug use. Additionally, long-term studies will also be necessary to evaluate if the alterations in neurotransmission and social cognition reported in Chapter 3 and 4 are reversible after the cessation of drug use.

We did not find a correlation between self-reported cocaine abstinence duration and startle reactivity or PPI. However, previous studies have been inconsistent regarding the influence of abstinence duration on startle reactivity: Efferen *et al.* (2000) reported decreased startle reactivity in early abstinent cocaine users, whereas Corcoran *et al.* (2011) only found reduced startle magnitude after 40 days of abstinence. In our study, which took place in an ambulant setting, we had to rely on self-reported abstinence duration beyond the period that can be controlled by urine toxicology. Therefore, further studies are needed to disclose the effect of abstinence on startle reactivity. Furthermore, our data suggest that ADHD symptoms might be associated with stronger craving and a higher vulnerability to cocaine-induced plasticity. These relationships have to be investigated more specifically, especially as drug users with ADHD symptoms may represent a clinical subpopulation which might have special needs in therapy and relapse prevention. Moreover, as PPI and also the vulnerability to develop substance abuse disorders have, at least partly, a genetic basis (Agrawal and Lynskey, 2008; Anokhin *et al.*, 2003; Hasenkamp *et al.*, 2010; Quednow *et al.*, 2010), genetic influences on PPI in drug users should be investigated.

Finally, we reported that cocaine users display specific deficits in social cognition which are related to real-life social functioning. These data corroborate the model by Volkow *et al.* (2011a) (Chapter 1.1). Social cognition deficits have also been shown in alcohol dependent patients (Uekermann *et al.*, 2007) and proposed in methamphetamine users (Homer *et al.*, 2008). However, to fully generalize this model, social cognition has to be investigated in other legal and illegal drugs. Furthermore, our data implicate that brain areas related to emotional empathy are more strongly and earlier influenced by cocaine use than areas related to cognitive empathy and mentalizing. The application of imaging methods ought to be considered to further investigate this hypothesis. Moreover, as reward systems have been shown to be impaired in drug users (Goldstein *et al.*, 2007; Jentsch and Taylor, 1999; Volkow *et al.*, 2010), the deficits in social cognition may also arise from a different processing of social

rewards and therefore a reduction in motivation for social interaction. This hypothesis is currently being investigated by our group using functional magnetic resonance imaging. More precisely, an interactive eye-tracking paradigm is applied to investigate the neural correlates of social gaze behavior and joint attention in cocaine users and controls. It has previously been reported that self-initiated joint attention is experienced as pleasant and accompanied by an increase in neural activity in reward related brain areas in healthy participants. This hedonic experience might underlie the human motivation to share experiences (Schilbach *et al.*, 2009). We hypothesize that joint attention is processed differently in cocaine users and is not associated with the same activations in the reward system as in controls. Furthermore, a second paradigm is applied to test the processing of social rewards through social agreement. It has been shown that the agreement with two "expert" reviewers on music choice leads to increased activity in the ventral striatum (Campbell-Meiklejohn *et al.*, 2012). Again, we expect cocaine users to be less rewarded by social agreement and therefore to show less activation in reward related brain areas than controls. These results would further corroborate the hypothesis that alterations in the reward system of cocaine users lead to altered processing of social reward and might explain reduced motivation for social interaction and deficits in social behavior in cocaine users.

Lastly, the application of comprehensive psychiatric diagnostics and hair toxicology has been proven to be a successful approach to collect a well-described sample of drug users without politoxic drug use patterns. Therefore, the application of methods to quantify drug use over a longer time period is strongly encouraged in future studies with drug users.

5.7 References

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Vonmoos M, Hulka LM, **Preller KH**, Jenni D, Baumgartner MR, Stohler R, Bolla KI, Quednow BB (2013): Cognitive dysfunctions in recreational and dependent cocaine users: The role of ADHD, craving, and early age of onset. *(submitted for publication).*

Vonmoos M, Hulka LM, **Preller KH**, Jenni D, Baumgartner M, Stohler R, Quednow BB (2013). Dissociation of self-report and behavioural measures of impulsive behaviour in cocaine users. *(submitted for publication).*

Hulka LM, **Preller KH**, Treyer V, Johayem A, Vonmoos M, Markus R. Baumgartner MR, Ametamey S, Buck B, Quednow BB (2013): Nicotine but not cocaine is associated with decreased metabotropic glutamate receptor 5 density in humans. *(submitted for publication).*

Hysek CM, Schmid Y, Simmler LD, Domes G, Heinrichs M, Eisenegger C, **Preller KH**, Quednow BB, Liechti ME (2013): Acute MDMA Enhances Emotional Empathy and Prosocial Behavior. *(submitted for publication).*

Preller KH, Schilbach L, Hulka LM, Vonmoos, M, Ingold N, Seifritz E, Herdener M, Quednow BB (2013): Impaired joint attention in recreational and dependent cocaine users – an fMRI study. *(in preparation).*

Invited Talks

Preller KH. Sustained incentive value of heroin-related cues in short- and long-term abstinent heroin users. ISAM 2012, Geneva, 15.10.2012.

Poster Presentations

Preller KH, Hulka L, Jenni D, Vonmoss M, Dziobek I, Quednow BB. How empathetic are cocaine users? - A social neuroscience approach. *The International College of Neuropsychopharmacology (CINP), 28th World Congress, Stockholm, Sweden, 2012.*

Preller KH, Hulka L, Jenni D, Quednow BB. Veränderungen der geteilten Aufmerksamkeit bei Kokainkonsumenten. *DGPPN, Berlin, Germany, 2011.*

Preller KH, Schilbach L, Hulka L, Jenni D, Quednow BB. Impaired joint attention in cocaine users. *24th Congress of the European College of Neuropsychopharmacology (ECNP), Paris, France, 2011.*

Preller KH, Hulka L, Jenni D, Vonmoss M, Dziobek I, Quednow BB. Emotional processing in cocaine users, *ZNZ Symposium, Zurich, Switzerland, 2011.*

Preller KH, Schilbach L, Hulka L, Jenni D, Quednow BB. Impaired joint attention in cocaine users. *Psychologie und Gehirn, 36. Arbeitstagung Psychophysiologische Methodik (APM), Heidelberg, Germany, 2011.*

Preller KH, Dörig N, Hasler F, Vollenweider FX, Quednow BB. Serotonergic challenge of cognitive functions in ecstasy users. *23rd ECNP Congress, Amsterdam, Netherlands, 2010.*

Preller KH, Hulka L, Jenni D, Quednow BB. Impaired joint attention in cocaine users. *ZNZ symposium 2010, Zurich, Switzerland, 2010.*

Awards

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